09/674035
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Practitioner's Docket No. 22727/04050A **PATENT**

Preliminary Classification:
Proposed Class:
Subclass:

NOTE: *"All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P. § 601, 7th ed.*

TRANSMITTAL LETTER TO THE U.S. DESIGNATED OFFICE (DO/US)— ENTRY INTO THE U.S. NATIONAL STAGE UNDER CHAPTER I

PCT/IB99/01818	11 November 1999	26 February 1999
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

DETECTING THE EXPRESSION OF THE DESC1 GENE IN SQUAMOUS CELL CARCINOMA
TITLE OF INVENTION

Jas C. Lang
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

ATTENTION: DO/US

NOTE: *The completion of those filing requirements that can be made at a time later than 20 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 U.S.C. § 371(d). The filing receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 C.F.R. § 1.491, which states: "An international application enters the national stage when the applicant has filed the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495."*

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date October 25, 2000, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL084655141US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Angela Suber
(type or print name of person mailing paper)

Angela Suber
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

WARNING:** *Each paper or fee filed by "Express Mail"* ***must *have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will ***not*** *be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

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WARNING: *Where the items are those that can be submitted to complete the entry of the international application into the national phase subsequent to 20 months from the priority date, the application is still considered to be in the international stage. And if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (because international application papers are not covered by an ordinary certificate of mailing. 37 C.F.R. § 1.8(2)(xi)).*

WARNING: *Documents and fees must be clearly identified as a submission to enter the national stage under 35 U.S.C. § 371, otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).*

WARNING: *Failure to pay the national fee within 20 months from the priority date will result in the abandonment of the application. The time for payment of the basic fee is not extendable. M.P.E.P. § 1893.01(a)(1), 6th ed., rev. 3.*

1. Applicant herewith submits to the United States Designated Office (DO/US) the following items under 35 U.S.C. § 371:

a. ☑ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☑ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and

☐ other fees (37 C.F.R. § 1.492), as indicated below:

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2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☐*	TOTAL CLAIMS	22 —20=	2	×$ 18.00=	$ 36.00
	INDEPENDENT CLAIMS	10 —3=	7	×$ ~~78.00~~ $80.00	$ 560.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	
BASIC FEE**	The international search fee, as set forth in § 1.445(a)(2) to be paid to the US PTO acting as an International Searching Authority: ☒ has been paid (37 CFR 1.492(a)(2)) .... ~~$690.00~~ $710.00 ☐ has not been paid (37 CFR 1.492(a)(3)) .... $970.00 ☐ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) $840.00				$710.00
	Total of above Calculations				= $1,306.00
SMALL ENTITY	Reduction by ½ for filing by small entity, if applicable. Affidavit must be filed also. (note 37 CFR 1.9, 1.27, 1.28)				– $653.00
	Subtotal				$653.00
	Total National Fee				$ 653.00
	Fee for recording the enclosed assignment document $40.00 (37 CFR 1.21(h)). (See Item 10 below). See attached "ASSIGNMENT COVER SHEET (37 C.F.R. § 3.34)".				
TOTAL	Total Fees enclosed				$ 653.00

* See attached Preliminary Amendment Reducing the Number of Claims.

****WARNING:*** *"To avoid abandonment of the application, the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 20 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 20-month time limit may not be extended." 37 C.F.R. § 1.494(b).*

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i. ☒ A check in the amount of $ 653.00 to cover the above fees is enclosed.

ii. ☐ Please charge Account No. ________ in the amount of $ ________________.

A duplicate copy of this sheet is enclosed.

WARNING: *If the translations of the international application and/or the oath or declaration have not been submitted by the applicant within twenty (20) months from the priority date, the applicant will be so notified and given a period of time within which to file the translation and/or oath or declaration in order to prevent abandonment. The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than twenty (20) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than twenty (20) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 will apply. 37 C.F.R. § 1.494(c).*

3. A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

a. ☒ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☐ has been transmitted

i. ☐ by the International Bureau. Date of mailing of the application (from form PCT/IB/308): ________________.

ii. ☐ by applicant on ________________
Date

NOTE: Section 1.494(b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 20 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies the applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage and applicant has received notice from the International Bureau, applicant need only pay the basic national fee by 20 months from the priority date." [This can now be paid subsequently with a surcharge.] Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35.

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on ________________
Date

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: *The Notice of January 7, 1993 indicates that 37 C.F.R. § 1.494(d) was "amended to clarify the existing practice that PCT Article 19 Amendments must be submitted by 20 months from the priority date, which time may not be extended." This Notice further advises: "Of course, the failure to do so does not result in loss of the subject matter of PCT Article 19 amendments. The applicant may submit that subject matter in a preliminary amendment filed under Section 1.121. In many cases, filing an amendment under Section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 35. See item 11(c) below. See also 37 C.F.R. § 1.494(d).*

a. ☒ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau. Date of mailing of the amendment (from form PCT/IB/308): __________.

ii. ☐ by applicant on __________ (Date)

c. ☐ have not been transmitted, as

i. ☐ no notification has been received that the International Search Authority has received the Search Copy.

ii. ☐ the Search Copy was received by the International Searching Authority, but the Search Report has not yet been issued. Date of receipt of Search Copy (from form PCT/ISA/202): __________.

iii. ☐ applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210): __________.

iv. ☐ the time limit for the submission of amendments has not yet expired. The amendments, or a statement that amendments have not been made, will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the amendments were made in the English language.

c. ☐ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ An oath or declaration of the inventor, including power of attorney, (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on __________ (Date)

b. ☐ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or (c) and 5(b); and states that they were reviewed by the inventor, as required by 37 C.F.R. § 1.70.

iii. ☒ will follow.

Other document(s) or information included:

8. ☒ An international Search Report or Declaration under PCT Article 17(2)(a):
   - a. ☐ is transmitted herewith.
   - b. ☐ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): ______________.
   - c. ☒ is not required, as the application was searched by the United States International Searching Authority.
   - d. ☐ will be transmitted promptly upon request.
   - e. ☐ has been submitted by applicant on ______________ Date
   - f. ☐ is not transmitted, as the international search has not yet issued.
9. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:
   - a. ☐ is transmitted herewith.

     Also transmitted herewith is (are)

     ☐ Form PTO—1449 (PTO/SB/08A and 08B)

     ☐ Copies of citations listed
   - b. ☒ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).
   - c. ☐ was previously submitted by applicant on ______________ Date
10. ☐ An assignment document is transmitted herewith for recording. A separate

    ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or

    ☐ FORM PTO—1595

    is also attached.

    ☐ Please mail the recorded assignment document to:

    i. ☐ the person whose signature and address appears below.

    ii. ☐ the following:

11. ☒ Additional documents
    - a. ☒ Copy of request (PCT/RO/101)
    - b. ☐ International Publication No. ______
        - i. ☐ Specification, claims and drawing
        - ii. ☐ Front page only
    - c. ☒ Preliminary amendment (37 C.F.R. § 1.121)
    - d. ☐ Other

12. ☒ The above checked items are being transmitted
    - a. ☐ before the 18th month publication.
    - b. ☒ after publication and the article 20 communication, but before 20 months from the priority date.
    - c. ☐ after 20 months (revival).

NOTE: Petition to revive (37 C.F.R. § 1.137(a) or (b)) is necessary if 35 U.S.C. § 371 requirements are submitted after 20 months.

13. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on ______________________ namely:
    Date

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 03-0172.

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 20 months without extension (37 C.F.R. § 1.494(b)(2)), results in abandonment of the application, it would be best to always check the above box.*

☐ 37 C.F.R. § 1.492(b), (c), and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment, prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☐ 37 C.F.R. § 1.17 (application processing fees)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a)).

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☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b)).

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying or at the time of paying . . . issue fee. . .." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 20 months after the priority date.

Pamela A. Docherty

Signature of practitioner

Reg. No. 40,591

Pamela A. Docherty

(type or print name of practitioner)

Tel. No.: (216) 622-8416

1400 McDonald Investment Center
800 Superior Avenue

P.O. Address

Customer No.:

Cleveland, Ohio 44114

(Transmittal Letter to the United States Designated Office (DO/US)—Entry into National Stage under 35 U.S.C. § 371 [13-6]—page 8 of 8)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	:	Lang
FOR	:	**DETECTING THE EXPRESSION OF THE DESC1 GENE IN SQUAMOUS CELL CARCINOMA**
SERIAL NO.	:	Not yet assigned
FILED	:	Concurrently herewith
ATTORNEY DOCKET NO.	:	22727/04050A

37 C.F.R. 1.27 VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS-NONPROFIT ORGANIZATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Ohio State University Research Foundation
ADDRESS: 1960 Kenny Road
Columbus, Ohio 43210-1063

TYPE OF ORGANIZATION

- ☐ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
- ☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3))
- ☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
  (name of state ____________________)
  (citation of statute ________________)
- ☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
- ☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
  (name of state ____________________)

(citation of statute ________________)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled:

DETECTING THE EXPRESSION OF THE DESC1
GENE IN SQUAMOUS CELL CARCINOMA

by inventors: Jas C. Lang

described in

- ☒ the specification filed herewith.
- ☐ application Serial No. ______________, Filed: ______________
- ☐ Patent No. ________________,
- ☐ issued ________________.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

* Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING	**Robin L. Rasor**
TITLE IN ORGANIZATION	Director, Technology Licensing
ADDRESS OF PERSON SIGNING	1960 Kenny Road Columbus, Ohio 43210-1063

SIGNATURE ____________________ DATE 25 Oct '00
Robin L. Rasor

{WAF6415;1}

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EXPRESS MAILING CERTIFICATE
"EXPRESS MAIL" Mailing Label No.: EL084655141US
Date of Deposit : October 25, 2000
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Box PCT, Assistant Commissioner for Patents, Washington, D.C., Attn: DO/US.
Typed or printed name of person signing this certificate: Angela Suber

Signed: Angela Suber

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Lang	)	Examiner: Not yet assigned
Serial Number: Not yet assigned	)	
International Application No.: PCT/IB99/01818	)	Art Unit: Not yet assigned
Filed: Concurrently herewith	)	
International Application Filed: November 11, 1999	)	
For: **Detecting the Expression of the DESC1 Gene in Squamous Cell Carcinoma**	)	Attorney Docket No.: 2727/04050A

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231
Attn: DO/US

PRELIMINARY AMENDMENT

Dear Sir:

Please preliminarily amend the above-identified application as follows:

IN THE SPECIFICATION

Page 1, before "Background of the Invention" insert ---

Cross-Reference to Related Applications

This application is a continuation of copending International Application PCT/IB99/01818, filed on November 11, 1999 and which designated the U.S. The non-provisional application designated above, namely International Application PCT/IB99/01818,

filed November 11, 1999, claims priority from U.S. Provisional Application 60/122,747, filed February 26, 1999.

IN THE CLAIMS

Please amend the claims as follows:

5. (Once Amended) A recombinant host cell comprising the recombinant [vecotor] vector of claim 4.

9. (Once Amended) The isolated nucleic acid of claim 2 wherein the [polynucleotide] nucleic acid hybridizes under stringent conditions with the nucleotide sequence shown in Figure 1A or 1B.

Please add the following claims:

10. (New) A method for diagnosing squamous cell carcinoma in a subject, comprising:

determining the presence, absence, or amount of expression of the DESC1 gene in a tissue sample obtained from the subject, wherein the diagnosis of squamous cell carcinoma is based on the presence, absence, or amount of expression of the DESC 1 gene in the sample.

11. (New) The method of claim 10 wherein the tissue sample is an epithelial tissue sample from the head, neck, oral mucosa, tonsils or skin of the subject.

12. (New) The method of claim 10 wherein the level of DESC 1 gene expression is determined using a nucleic acid probe which hybridizes to a transcript of the DESC 1 gene.

13. (New) The method of claim 10 wherein the level of expression of the DESC 1 gene is determined using a polymerase chain reaction and primers which are complementary to specific regions of the DESC 1 gene.

14. (New) The method of claim 10 wherein the level of expression of the DESC 1 gene is determined by assaying for the presence, or absence, or a change in the levels of the protein encoded by the DESC 1 gene in the sample.

15. (New) The method of claim 14 wherein an antibody which is immunospecific for the protein encoded by the DESC 1 gene is employed in the assay.

16. (New) A method for diagnosing prostate carcinoma in a subject, comprising:

determining the presence of, or absence of, or amount of expression of the DESC1 gene in a tissue sample obtained from the prostate of the subject, wherein the diagnosis of prostate carcinoma is based on the presence, absence, or amount of expression of the DESC 1 gene in the sample.

17. (New) The method of claim 16 wherein the level of DESC 1 gene expression is determined using a nucleic acid probe which hybridizes to a transcript of the DESC 1 gene.

18. (New) The method of claim 16 wherein the level of expression of the DESC 1 gene is determined using a polymerase chain reaction and primers which are complementary to specific regions of the DESC 1 gene.

19. (New) The method of claim 16 wherein the level of expression of the DESC 1 gene is determined by assaying for the presence, or absence, or a change in the levels of the protein encoded by the DESC 1 gene in the sample.

20. (New) The method of claim 19 wherein an antibody which is immunospecific for the protein encoded by the DESC 1 gene is employed in the assay.

21. An isolated polynucleotide which encodes amino acids 191 through 422 of the amino acid sequence shown in Figures 1A or 1B.

22. A DESC 1 polypeptide which comprises a sequence which is 95% identical to the sequence extending from and including amino acid 191 through amino acid 422 of the amino acid sequence shown in Figures 1A or 1B.

REMARKS

By the present amendment, the specification is amended to claim priority from related international and provisional applications. By the present amendment, original claims 5 and 9 are amended to correct typographical errors. By the present amendment, new claims 10-22 are added to the application. Support for new claims 10-22 is found in paragraph 1 of the Summary of the Invention. Support for new claims 10-20 is also found in paragraph 1 of the Detailed Description of the Invention, pages 10-11 and 13, and Examples 1, 2, 3, and 5. Support for new claims 21 and 22 is found in the first paragraph on page 7. Applicant also submits herewith an amended specification which was sent to the ISA/USA on February 22, 2000. The amended

specification has the figures which were originally incorporated into the text moved to the end of the document. The amended specification also a the brief description of the figures that had originally been in the text of the application has also been incorporated into the Brief Description of the Figures section of the amended version of the application. The amendments and new claims add no new matter to the application.

Applicant respectfully requests that the present Preliminary Amendment be entered.

Respectfully submitted,

Date: October 25, 2007

By: Pamela A. Docherty

Pamela A. Docherty, Reg. No. 40,591
CALFEE, HALTER & GRISWOLD LLP
1400 McDonald Investment Center
800 Superior Avenue
Cleveland, Ohio 44114
(216) 622-8416

DETECTING THE EXPRESSION OF THE DESC1 GENE IN SQUAMOUS CELL CARCINOMA

This invention was made in part with government support under CA63134 and DE12704 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

Background of the Invention

Typically, morphological examination of the tissues is performed to characterize squamous cell carcinomas of the head and neck and tissues adjacent to such tumor tissue. The morphological examination usually involves the sectioning and staining of an excised tissue sample followed by microscopic examination by a cytologist or pathologist. However, visual examination does result in some errors, particularly by pathologists who do not routinely encounter such tissue samples.

Accordingly, it is desirable to have a technique which does not require visual examination of morphological characteristics to diagnose or confirm a pathologist's diagnosis of squamous cell carcinoma of the head and neck.

Summary of the Invention

The present invention provides a novel method for diagnosing squamous cell carcinoma or prostate cancer in a tissue sample. The method does not involve visual examination of morphology. The method comprises providing a sample from the subject and assaying for the presence, or absence or reduced level of expression of a novel gene, hereinafter referred to as the "DESC1 gene". The method comprises isolating RNA, preferably mRNA from tissue samples, and detecting the mRNA which encodes all or part of DESC1 protein. Preferably the detection comprises amplifying the mRNA, preferably by reverse transcriptase-PCR using primers specific to a region in the DESC1 gene; and detecting the presence or absence of the amplified product to determine whether DESC1 mRNA is present or absent in the tissue sample. Alternatively the detection comprises separating the RNA which encodes all or part of DESC1 protein from the total RNA, preferably by separating on an agarose gel, and detecting the mRNA encoding DESC1, preferably by using a probe specific for such mRNA. Optionally, the DESC1 mRNA when present, is also quantified using conventional techniques.

The present invention also relates to polynucleotides which encode the DESC1 protein, to the DESC 1 protein, and to antibodies to the DESC1 protein. The present invention also relates to hybridization probes, and to primers useful in the method of detecting DESC1 mRNA.

Brief Description of the Figures

FIGURE 1A is the nucleotide sequence, SEQ.ID.NO. 1, of a cDNA which encodes a DESC1 protein, with the predicted amino acid sequence of the amino acid sequence, SEQ. ID. NO. 2 encoded by the nucleotide sequence. Putative initiation and termination codons are boxed, as are residues predicted to represent conserved amino acids of the catalytic triad. Suggested location of catalytic cleavage site is shown by vertical arrow. Consensus polyadenylation signals are double underlined. The predicted hydrophobic transmembrane sequence or signal peptide is located at about amino acids 18-37 and is underlined.

FIGURE 1B is an alternate nucleotide sequence, SEQ.ID.NO. 3, of a cDNA which encodes a DESC1 protein, with the predicted amino acid sequence of the amino acid sequence, SEQ. ID. NO. 4 encoded by the nucleotide sequence.

Figure 2. DESC1 expression in normal tissue and metastatic neck node used for RDA analysis and in primary carcinoma from the same patient. RT-PCR analysis. Primers utilized: D10, D11. Size of RT-PCR product, 555 bp.

Figure 3. Expression of DESC1 in squamous cell carcinoma of the head and neck (SSCHN) and matched normal tissues. A, RT-PCR analysis of DESC1 expression in SCCHN and matched normal tissue. Primers utilized: D3, D4. Size of RT-PCR product 149 bp. Low molecular weight band seen in negative control lane and some sample lanes represents unincorporated [α^{32}P]dCTP. *B*, Northern analysis of DESC1 expression in SCCHN, matched normal tissue and metastatic neck node. PHA, pharynx; TSL, tonsil; FOM, floor of mouth; TON, tongue; EPI, epiglotis; LAR, larynx; R T, retromolar trigone; T B, tongue base.

Figure 4. Expression of DESC1 in epithelial cell lines and human tissues. RT-PCR analysis using total RNA derived from epithelial cell lines and human tissues. Primers utilized, D10 and D11.

Figure 5. Expression of DESC1 in multiple human tissues. Northern analysis using *A* total RNA (Human Normal Tissue Blot III) and *B*, polyadenylated RNA (Human Multiple Tissue Northern Blot II)

Figure 6. Expression of DESC1 in prostate cancer specimens and clel lines. RT-PCR analysis of (A) prostate cancer xenografts shown in mice and (B) human prostate cell lines

Figure 7. Expression of DESC1 in transfected human embryonal kidney epithelial cells. Western Analysis of lysates from cells

Figure 8. Protease Activity of recombinant DESC1 protein purified from transfected COS cells.

Detailed Description of the Invention

A novel gene has been discovered which is expressed in significant levels in epithelial derived tissues, specifically epithelial tissues of the head, neck, oral mucosa, tonsils, prostate, testes, and skin in healthy individuals, i.e. individuals who do not have squamous cell carcinoma or prostate carcinoma. However, in tumor tissue samples taken from patients with squamous cell carcinoma, particularly of the head and neck, the expression of the DESC1 gene is absent or significantly reduced. The differential expression permits the identification of squamous cell carcinoma of the head and neck.
Similarly, expression of the DESC1 gene is reduced or absent in prostate carcinoma and, thus, permits identification of prostate carcinoma.

Polynucleotides

The present invention provides isolated polynucleotides which encode a DESC1 protein. One embodiment of a polynucleotide which encodes a DESC1 protein is shown in Fig. 1A, another embodiment is shown in Fig. 1B

Due to the known degeneracy of the genetic code wherein more than one codon can encode the same amino acid, a DNA sequence may vary from that shown in Fig 1A and still encode a DESC1 protein having the amino acid sequences shown in Fig. 1A. Similarly, a DNA sequence may vary from that shown in Fig. 1A and 1B and still encode the amino acid sequence shown in Fig. 1B.

The present invention also encompasses polynucleotides having sequences that are capable of hybridizing to the nucleotide sequences of Figs 1A and 1B under stringent conditions, preferably highly stringent conditions. Hybridization conditions are based on the melting temperature TM of the nucleic acid binding complex or probe, as described in Berger and Kimmel (1987) Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 152, Academic Press. The term "stringent conditions, as used herein, is the "stringency"

which occurs within a range from about Tm-5 (5° below the melting temperature of the probe) to about 20° C below Tm. . "Highly Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.2x SSC at about 65 degree C. As recognized in the art, stringency conditions can be attained by varying a number of factors such as the length and nature, i.e., DNA or RNA, of the probe; the length and nature of the target sequence, the concentration of the salts and other components, such as formamide, dextran sulfate, and polyethylene glycol, of the hybridization solution. All of these factors may be varied to generate conditions of stringency which are equivalent to the conditions listed above.

Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lower stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2 M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

The present invention also encompasses alleles of the DESC1 protein encoding sequences. As used herein, the term an "allele" or "allelic sequence" is an alternative form of an DESC1 encoding sequence. The allele may result from one or more mutations in the DESC1 encoding sequence. Such mutations typically arise from natural addition, deletion of substitution of nucleotides in the open reading frame sequences. Any gene which encodes a DESC1 protein may have none, one, or several allelic forms. Such alleles are identified using conventional techniques, such as, for example, screening libraries with probes.

The present invention also encompasses altered polynucleotides which encode a DESC1 protein. Such alterations include deletions, additions, or substitutions. Such alterations may produce a silent change and result in a DESC1 protein having the same amino acid sequence as the DESC1 protein encoded by the unaltered polynucleotide. Such alterations may produce a nucleotide sequence possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eucaryotic host may be incorporated into the nucleotide sequences shown in Figs 1A and 1B to increase the rate of expression of the polypeptides encoded by such sequences. Such alterations, conventionally, are accomplished using site-directed mutagenesis.

The polynucleotides are useful for producing DESC1 protein. For example, an RNA molecule encoding a DESC1 protein is used in a cell-free translation systems to prepare such polypeptide. Alternatively, a DNA molecule encoding a DESC1 protein is introduced into an expression vector and used to transform cells. Examples of expression vectors are chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40, bacterial plasmids, phage DNAs; yeast plasmids, vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, baculovirus, and retrovirus. The DNA sequence is introduced into the expression vector by conventional procedures.

Accordingly, the present invention also relates to recombinant constructs comprising one or more of the polynucleotide sequences. Examples of constructs are vectors, such as a plasmid, phagemid, or viral vector, into which a sequence that encodes the DESC1 protein has been inserted. In the expression vector, the DNA sequence which encodes the DESC1 protein is operatively linked to an expression control sequence, i.e., a promoter, which directs mRNA synthesis. Representative examples of such promoters, include the LTR or SV40 promoter, the *E. coli* lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or in viruses. The promoter may also be the natural promoter of the DESC1 encoding sequence. The expression vector, preferably, also contains a ribosome binding site for translation initiation and a transcription terminator. Preferably, the recombinant expression vectors also include an origin of replication and a selectable marker, such as for example, the ampicillin resistance gene of *E. coli* to permit selection of transformed cells, i.e. cells that are expressing the heterologous DNA sequences. The polynucleotide sequence encoding the DESC1 protein is incorporated into the vector in frame with translation initiation and termination sequences.

The polynucleotides encoding a DESC1 protein are used to express recombinant protein using conventional techniques. Such techniques are described in Sambrook, J. et al (1989) Molecular Cloning A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y. and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John wile & Sons, New York, NY.

Polynucleotides encoding a DESC1 protein or fragments thereof, are also useful for designing hybridization probes for isolating and identifying cDNA clones and genomic clones encoding a DESC1 protein or allelic forms thereof. Such hybridization techniques are conventional. The sequences that encode the DESC1 proteins or fragments thereof, are also useful for designing primers for polymerase chain reaction (PCR), a technique useful for obtaining large quantities of cDNA molecules that encode the DESC1 proteins.

Also encompassed by the present invention, are single stranded polynucleotides, hereinafter referred to as antisense polynucleotides, having sequences which are complementary to the DNA and RNA sequences which encode the DESC1 proteins. The term "complementary" as used herein refers to the natural binding of the polynucleotides under permissive salt and temperature conditions by base pairing.

Isolated polynucleotides encoding a DESC1 protein are also useful as chromosome markers to map related gene positions. DESC1 polynucleotide probes are preferably labeled with radioisotopes, fluorescent labels or enzymatic labels.

Polynucleotides encoding an DESC1 protein are useful to detect DESC1 gene expression in biopsied tissue samples. DESC1 polynucleotides or fragments thereof are also useful as probes or primers to identify tissues or cells in which the corresponding DESC1 gene transcript is expressed.

Proteins

The DESC1 protein possesses four regions of conserved homology with members of the serine protease gene family. The sequence identity between DESC1 protein and the serine protease, Human Airway Trypsin-like Protease (HAT)(Fig. 1B) at the amino acid level is 38% overall and 51% when the serine protease catalytic domain only is compared (DESC1 residues 191-422). This information suggests that DESC1 protein is a novel member of the serine-protease gene family.

DESC 1 protein has the following conserved domains: (a) a predicted hydrophobic transmembrane domain located at about amino acids 18-37; (b) a predicted catalytic cleavage site located at about amino acids 190-191; and (c) a predicted catalytic domain located at about amino acids 191-422, containing conserved residues comprising the serine protease

catalytic triad at about amino acids 231 (histidine), 276 (aspartic acid) and 372 (serine). As shown in Figure 1, the predicted mature protein encompasses about 422 amino acids, while the predicted secreted and cleaved form of DESC1, which may be membrane-bound or soluble, encompasses about 232 amino acids (residues 191-422).

DESC1 may, similar to HAT, be a transmembrane serine protease possessing an extracellular COOH-terminal catalytic region. Accordingly, DESC1 protein may be used to cleave naturally occurring substrate proteins and by amino acid substitutions, to cleave proteins which are substrates of other serine proteases. Thus DESC1 polypeptides can be used to cleave a peptide for usage in microsequencing or for peptide mapping of proteins. DESC1 serine protease activity may be assayed utilizing standard methodologies used to demonstrate the activity of other serine proteases, as described for example by Smyth et al., J. Biol. Chem., 267: 24418-24425 and utilizing commercially available serine protease substrates including, but not limited to, Benzoyl-prolyl-phenyl-alanyl-arginine-4-nitril-anilide acetate; Tosyl-glycyl-prolyl-lysine-4-ntranilide acetate; Carbobenzoxy-valyl-glycyl-arginine-4-nitril-analide acetate and N-Methoxycarobonyl-D-norleucyl-glycl-L-arginine-4-nitranilide acetate (Boehringer Mannheim). Additionally, assay of serine protease activity of DESC1 can be utilized to identify inhibitors of DESC1 activity, by addition of known protease inhibitors to the assay, such as alpha2-macroglobulin, 2-(2-Aminoethyl)-benzyensulfonyl fluoride hydrochloride and Leupeptin (Boehringer Mannheim).

The term DESC1 protein in addition to encompassing the amino acid sequences of the reference amino acid sequences shown in Figures 1A and 1B, also encompasses variant DESC1 proteins whose amino acid sequence is similar to one of the reference amino acid sequences, but does not have 100% identity with the reference amino acid sequences. Such variant DESC1 protein has an altered sequence in which one or more of the amino acids is deleted or substituted, or one or more amino acids are inserted, as compared to the reference amino acid sequence. Such variant DESC1 proteins have an amino acid sequence which is at least 90% identical, preferably, at least 95% identical, more preferably at least 98% identical, most preferably at least 99% identical to the reference amino acid sequence. Sequences which are at least 90% identical have no more than 5 alterations, i.e. any combination of deletions, insertions or substitutions, per 100 amino acids of the reference amino acid sequence. Percent identity may be determined by comparing the amino acid sequence of the variant DESC1 protein with the reference sequence using MEGALIGN project in the DNA STAR program. The variant sequences and reference sequences are aligned for identity calculations using the method of the software basic local alignment search tool in the BLAST

network service (the National Center for Biotechnology Information, Bethesda, MD) which employs the method of Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403-410. Identities are calculated, for example, by the Align program (DNAstar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are not ignored when making the identity calculation.

While variant DESC1 proteins have non-conservative amino acid substitutions, it is preferred that variant DESC1 proteins have the conservative amino acid substitutions. In conservative amino acid substitutions, the substituted amino acid has similar structural or chemical properties with the corresponding amino acid in the reference sequence. By way of example, conservative amino acid substitutions involve substitution of one aliphatic or hydrophobic amino acids, e.g. alanine, valine, leucine and isoleucine, with another; substitution of one hydroxyl-containing amino acid, e.g. serine and threonine, with another; substitution of one acidic residue, e.g. glutamic acid or aspartic acid, with another; replacement of one amide-containing residue, e.g. asparagine and glutamine, with another; replacement of one aromatic residue, e.g. phenylalanine and tyrosine, with another; replacement of one basic residue, e.g. lysine, arginine and histidine, with another; and replacement of one small amino acid, e.g., alanine, serine, threonine, methionine, and glycine, with another.

Preferably, the variant DESC1 protein is immunoreactive with antibodies that bind to the reference DESC1 protein. Guidance in determining which amino acid residues may be substituted, inserted or deleted without abolishing immunoreactivity of the variant protein with an antibody specific for the respective reference protein are found using computer programs well known in the art, for example, DNASTAR software.

The present invention also relates to fusion proteins comprising a DESC1 protein and a tag, i.e., a second protein or one or more amino acids, preferably from about 2 to 65 amino acids, more preferably from about 34 to about 62 amino acids, which are added to the amino or carboxy terminus of the DESC1 PROTEIN. Typically, such additions are made to stabilize the fusion protein or to simplify purification of an expressed recombinant form of the corresponding DESC1 protein Such tags are known in the art. Representative examples of such tags include sequences which encode a series of histidine residues, the epitope tag FLAG, the Herpes simplex glycoprotein D, beta-galactosidase, maltose binding protein, or glutathione S-transferase.

The present invention also encompasses DESC1 proteins in which one or more amino acids, preferably no more than 10 amino acids, are altered by post-translation processes or

synthetic methods. Examples of such modifications include, but are not limited to, acetylation, amidation, ADP-ribosylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or a lipid, cross-linking gamma-carboxylation, glycosylation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, sulfation, and transfer-RNA mediated additions of amino acids to proteins such as arginylation and ubiquitination.

The DESC1 protein and fragments thereof, particularly extracellular fragments thereof, are useful as immunogens to produce antibodies immunospecific for the DESC1 protein. The term "immunospecific" means the antibodies have substantially greater affinity for the DESC1 protein than for other proteins. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, and Fab fragments. Polyclonal antibodies are generated using conventional techniques, such as by administering the DESC1 protein or fragment thereof to a host animal. Depending on the host species, various adjuvants are preferably used to increase immunological response. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin, and Corynebacterium parvum are especially preferable). Conventional techniques are also used to collect blood from the immunized animals and to isolate the serum and/or the IgG fraction from the blood.

For preparation of monoclonal antibodies, conventional hybridoma techniques are used. Such antibodies are produced by continuous cell lines in culture. Suitable techniques for preparing monoclonal antibodies include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV hybridoma technique.

Various immunoassays may be used for screening to identify antibodies having the desired specificity. These include conventional techniques which involve competitive binding or immunoradiometric assays and typically involve the measurement of complex formation between the DESC1 protein and the antibody.

Antibodies to the DESC1 protein

Antibodies which are specific for and bind to the DESC1 protein or the extracellular domain of the DESC1 protein, are useful research tools for identifying tissues that contain reduced levels of the DESC1 protein and also for purifying the DESC1 protein, from cell or tissue extracts, or medium of cultured cells, or partially purified preparations of intracellular and extracellular protein. Such purification is accomplished by conventional techniques such as by affinity chromatography.

Method of Assaying for DESC1 mRNA in Tissue Samples

Expression of the DESC1 transcript in a tissue sample or cell sample is determined using conventional procedures including, but not limited to DNA-RNA hybridization or PCR amplification.

A sample of the subject's tissue is obtained from a site which is suspected as being a tumor site. Preferably, one or more tissue samples from the area adjoining or preferably distal to the putative tumor site are also obtained from the subject. More preferably, samples are also obtained from matched normal, unaffected epithelial tissue of the subject. Preferably, all tissue samples are snap-frozen in liquid nitrogen immediately following resection.

RNA is isolated from the tissue samples by conventional techniques such as a TRIzol extraction procedure see for example, Gramza et. al. "Efficient Method for Preparing Normal and Tumor Tissue for RNA Extraction" BioTechniques, volume 18, page 218 (1995) which is fully incorporated herein by reference.

The presence of DESC1 mRNA is detected by RT-PCR technology using a forward primer that anneals to a region on the antisense strand of the DESC1 gene and reverse primer which anneal to a region on the sense strand of the DESC1 gene. Preferably, the forward and reverse primers anneal to regions of the DESC1 gene which are separated by between 149 to 1471 base pairs, more preferably 300 to 400 base pairs. Preferably, the primers comprise 18-36 nucleotides, more preferably 22-31 nucleotides. Preferably, the primers have a G+C content of 40% or greater. More preferably the forward and reverse primers comprise the following sequences, respectively:

DESC1 antisense , D11, 5'-TGCATCAAGCAAACAGTTTATTGAGATC-3';

DESC1 sense, D10, 5'CCTGTTCCCTACACAAATHCAGTAC-3'.

The size of the PCR product produced using such primers is 555 bp.

Optionally, a normalization standard, such as a housekeeping gene, is included in the PCR reaction. The expression of the preferred housekeeping gene is the same level in normal squamous cells and in cells derived from squamous cell carcinoma tumor tissue. For example, suitable housekeeping genes are HPRT or genes encoding actin or tubulin. Primers to the normalization standard are selected such that the length of the PCR amplification product of the normalization standard will vary from the length of the PCR amplification product of DESC1 mRNA to allow separation of the two PCR amplification products on an agarose gel.

Preferably, an internal standard comprising a double-stranded nucleic acid fragment which competes with the DESC1 gene for the primers is added to the PCR reaction mixture. Preferably, the PCR product that results from amplification of the internal standard is of a different size from that of the PCR product that results from amplification of the DESC1 mRNA to enable separation of the two products on an agarose gel.

Optimum cycle number for PCR amplification is preferably pre-determined for each primer set using a mixture of RT reactions from ten random samples selected from specimens of tumor RNA and matched normal RNA. This step is necessary to ensure that PCR amplification remains in the linear range and that production of PCR product does not plateau. Under the reaction conditions used, the quantity of PCR product is directly proportional to the amount of radioactivity incorporated into the DNA. This method allows a comparative analysis of gene expression between samples through direct comparison of radioactivity incorporated into each PCR product.

Preferably, the presence of the primer-specific PCR product is detected by separating the PCR products on an agarose gel. The presence of the primer-specific product is detected by ethidium bromide staining of the agarose gel. More preferably, the primer-specific product contains alpha-^{32}P-deoxynucleotide as a result of incorporating alpha-^{32}-p-deoxynucleotide into the PCR reaction mixture. Detection of such PCR product is accomplished by measuring the amount of radiolabeled deoxynucleotide incorporated into the PCR product by gel scanning using autoradiograms, or by liquid scintillation counting of excised portions of the gel. Band intensity of DESC1 RT-PCR product is then compared and intensity is directly proportional to the level of expression of DESC1 RNA in the original tissue sample.

Alternatively the mRNA is extracted, separated, preferably on an agarose gel, and the mRNA encoding DESC1 is detected preferably using a probe specific for such mRNA.

DESC1 RNA is expressed in normal human epithelial tissue. DESC1 RNA is also expressed in normal human epidermal keratinocytes undergoing exponential growth in tissue culture (NEHK cells; Clonetics, San Diego, CA). DESC1 RNA expression is reduced or absent in squamous cell carcinoma (SCC). Thus, in one embodiment, reduced DESC1 expression in neoplastic tissue of epithelial origin may be considered to be a diagnostic indicator of SCC.

<u>Isolation and Sequence Analysis of DESC1 cDNA</u>

Representational Difference Analysis (RDA) was performed on mRNA isolated from

the normal oral buccal mucosa and from an squamous cell carcinoma -positive metastatic neck node from an individual who presented with a primary squamous cell carcinoma of the tongue which was metastatic to regional neck nodes. Representational Difference Analysis was performed with carcinoma RNA as driver in the reaction, allowing selection of genes expressed in normal tissue but not in tumor tissue. Representational Difference Analysis (RDA) was performed by utilizing PCR-Select cDNA subtraction methodologies (CLONTECH, Palo Alto, CA) as described in the manufacturer's protocol. 0.5% of the Representational Difference Analysis final reaction was subject to PCR amplification. The PCR amplification products thus obtained were cloned directly into mammalian expression vector pCMV-Script (Stratagene, La Jolla, CA).

All recombinant clones were screened for inserts by PCR analysis and positive clones subjected to sequence analysis using vector-specific T3 primers. BLASTN sequence analysis was then performed, using the GenBank sequence database. One recombinant clone designated "C35", carrying a 581 bp insert, possessed an open reading frame spanning the full length of the clone. The putative gene represented by this clone was designated Differential Expressed in Squamous Cell Carcinoma Gene 1 (DESC1). The C35 clone was devoid of consensus polyadenylation signals. In order to obtain the 3' end of the gene, 3'RACE analysis was performed on the remaining normal tissue mRNA used previously for RDA analysis. Sequence analysis of 3'RACE products allowed identification of two consensus polyadenylation signal sequences separated by 633 bp (Fig. 1A). In order to obtain additional 5' sequence, 5'RACE was also performed.

A full-length DESC1 cDNA clone (pDESC1) was constructed following analysis of DESC1 sequence information obtained from overlapping pCMV-Script, 5' and 3' RACE clones. pDESC1 was generated by RT-PCR amplification of normal skin RNA (Invitrogen, Carlsbad, CA) utilizing DESC1 primers D11 and D12. primers D11 and D12 have the following sequence:

D11, 5'-TGCATCAAGCAAACAGTTTATTGAGATC-3';

D12, 5'TGACTTGGATGTAGACCTCGACCTTCAC-3'.

The PCR product was then cloned into TOPO TA cloning vector pCDNA3.1/V5/His-Topo (Invitrogen, Carlsbad, CA).The sequence of pDESC1 was determined by cycle sequencing using a Thermo Sequenase system (Amersham, Cleveland, OH), followed by electrophoresis using the CastAway sequencing system (Stratagene, La Jolla, CA) according to the manufacturer's instructions.

The full-length nucleotide sequence(SEQ ID NO:1) of one cDNA encoding DESC 1 protein is shown in Fig. 1A. An alternate full-length nucleotide sequence, SEQ.ID.NO. 3 is shown in Figure 1B. The cDNA comprises a contiguous sequence of 1461 nucleotides which encodes a predicted open reading frame of 422 or 423 amino acid residues (SEQ ID NO:2). The open reading frame begins at an N-terminal methionine located at nucleotide position 53 or 56, and ends at a stop codon at nucleotide position 1322. The predicted molecular weight of the DESC1 protein is about 44 kDa.

Tissue Expression of DESC1

In order to characterize the tissue-specific expression pattern of DESC1, Northern analysis was performed on total RNA from multiple human tissue samples and cell lines. The results are shown in Figure 5 and demonstrate a high degree of tissue specificity of DESC1 expression. Under these conditions two predominant transcripts of 2.2 kb and 1.6 kb in size were detected in tonsil tissue. Under these conditions no significant level of DESC1 expression was detected in any other tissue examined, that is thymus, appendix, lymph node, gall bladder, ovary, spleen, colon small intestine, leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney prostate and testes. However, when Northern analysis was performed with a higher degree of sensitivity, utilizing polyadenylated RNA rather than total RNA, DESC1 expression was detected in prostate and testes. A single 1.6 kb transcript is seen in testes. The three transcripts detected in prostate include a 1.6 kb transcript a 2.2 kb transcript and an additional transcript of approximately 4.4kb, which is observed exclusively in prostate tissue. In addition, expression of DESC1 was detected at a minimal level in pancreas. No expression of DESC1 was detected in thymus, appendix, lymph node, gall bladder, ovary, spleen, colon small intestine, leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, and kidney. Thus, the DESC1 gene is strongly tissue-specific in its pattern of expression.

To determine whether DESC1 expression was epithelial-specific, the levels of DESC1 transcripts in epithelial cell lines and tissue were determined using RT-PCR analysis. The results, shown in Fig 4 established that DESC1 is expressed in neonatal (NHEKNeo) adult (NHEKAd) and prostate (PrEC) epithelial cells, confirming that the expression of the DESC1 gene is epithelial specific. Figure 4 also shows DESC1 expression in human skin and confirms expression in prostate and testes.

The RT-PCR analysis utilized primers D10 and D11, specific to the 3' end of DESC1, and produced a product that does not encompass the internal polyadenylation consensus site. This analysis allows detection of DESC1 expression in testes. Since testes exhibits only the smaller 1.6 kb transcript, the data is consistent with identification of the 1.6 kb RNA as the

transcript which encodes the DESC1 polypeptide. A second 3' RACE product isolated demonstrates processing of a DESC1 RNA at the internal polyadenylation site (nucleotides 802-807). This transcript terminates at nucleotide 823 and demonstrates that the internal site is functional.

Chromosomal Mapping Using a DESC 1 probe.

Chromosomal mapping of DESC1 gene was performed using a Human/Rodent Somatic Cell Hybrid Panel from Oncor, Gaithersburg, MD, with hybridization conditions for DESC1 probe as described above. The DESC1 probe hybridized to only the lane containing human chromosome 4. Chromosomal mapping of DESC1 was additionally performed using the Genebridge 4 Radiation Hybrid Panel (Research Genetics Inc., Huntsville, AL) according to the manufacturer's instructions by PCR amplification using primers D11 and D18, with normal human placental DdnA (Sigma, St. Louis, MO) as template. D18 primer has the following sequence, 5'-GGAATAGTGAGCTCGGGAGATG-3'.

The chromosomal location of DESC1 was then determined by accessing Whitehead Institute/MIT Center for Genome Research radiation hybrid map of the human genome. DESC1 is located on the long arm of chromosome 4, positioned 20.21cR from marker WI-5548, and between markers D4S1619 and WI-7844. D4S1619 and WI-7844 have been mapped by FISH analysis to 4q12 and 4q13. Thus DESC1 is at 4q12 - 4q13 within a region about 10Mb in size

The following examples, which describe in greater detail the procedures for determining DESC1 gene expression levels in tissue samples, are intended to illustrate but not limit the procedures.

Example 1

Ten squamous cell carcinoma specimens, and matched normal tissue, selected from diverse sites in the head and neck region were obtained. All normal tissue specimens were harvested from clinically appearing normal tissue located at least 3 cm. from the tumor margin. RNA was extracted from the samples and subjected to RT-PCR analysis. of expression of DESC1 was conducted in the samples.

1.0 μg of total RNA was used for first strand cDNA synthesis in a total volume of 25 μl and reactions otherwise performed according to manufacturer's instructions (ProSTAR, Stratagene, La Jolla, CA). PCR amplification was performed in the presence of 2 units of Taq 2000 DNA polymerase (Stratagene, La Jolla, CA), with reaction conditions: 10mM Tris-HCI (pH 8.8), 50 mM KCI, 1.5 mM $MgCl_2$, 0.01% gelatin, 400 nM each primer, 200μM

dNTPs, and where appropriate, 0.25μl [α^{32}P]dCTP (3000Ci/mmol) in a final volume of 25μl. Separate reactions were performed for each primer pair with reaction conditions; 96°C 3min followed by 94°C 30s, 55°C 30's, 72°C 1 min for 31 cycles (HPRT) or 33 cycles (DESC1) and a final 5 min extension at 72°C. PCR amplification of full-length DESC1 was performed utilizing the above cycling conditions, with an additional 1 min extension time for each cycle at 72°C, and using the Advantage HF PCR kit (CLONTECH Laboratories Ind, Palo Alto, CA). PCR samples were then run through 22% agarose gels and presence of amplified product and correct product size verified by ethidium bromide fluorescence in the presence of 100 bp size markers (Gibco BRL, Gaithersburg, MD). PCR products generated were then electroblotted using a Bio-Rad Semi-Dri Electroblotter SD and transferred at 12V/110 mA for 10 min. The membrane was removed and exposed to BioMax film (Eastman Kodak, Rochester, NY). Primers utilized in PCR reactions comprise: hypoxanthine phosphoribosyl transferase (HPRT) primers HPt1, 5'-GTAATGACCAGTCAACA-3' and HPRT2, 5'-CCAGCAAGCTTGCGACCTTGACCA-3' and DESC1 primers

D3, 5'-TCACTGTTCATTATGTGAGATATAATCA-3';

D4, 5'-CACCATTGATTCAAGTCTCTGGCTCAT-3';

D10, 5'-CCTGTTCCCTACACAAATGCAGTAC-3';

D11, 5'-TGCATCAAGCAAACAGTTTATTGAGATC-3';

D12, 5'-TGACTTGGATGTAGACCTCGACCTTCAC-3' and

D18, 5'-GGAATAGTGAGCTCGGGAGATG-3'.

PCR amplification of the HPRT gene was performed as a control to demonstrate equal loading and to determine integrity of RNA. Primers sets were designed by computer analysis (Oligo 4.0; NBI, Hamel, MN) of available DNA sequences for each gene and, with the exception of set D11, D18, are intron-spanning precluding PCR amplification of any residual DNA present in RNA samples. Optimum cycle number for PCR amplification was pre-determined for each primer set using a mixture of RT reactions from ten random tumor samples. This step is necessary to ensure that PCR amplification remains in the linear range and that production of PCR product does not plateau. Under reaction conditions used, quantity of PCR product is therefore directly proportional to the amount of radioactivity incorporated into the DNA.

The results, shown in Figure 3A, showed lower levels of expression of DESC1 in 9 of the 10 primary carcinomas relative to the high level of expression in matched normal tissue.

It has been discovered that in non cancerous epithelial tissue, DESC1 is expressed at a high level, yet in squamous cell carcinoma, DESC1 is not expressed or only expressed at a very low level, i.e., at levels less than 10% of the levels found in matched normal tissue

Example 2

Six specimens of squamous cell carcinoma of the head and neck, matched normal tissue and metastatic regional neck nodes were obtained. The mRNA extraction was conducted as in Example 1. A northern blot containing squamous cell carcinoma specimens was generated by electrophoresis of 10 μg total RNA on a 1% glyoxal agarose gel according to manufacturer's instructions (Northern Max-Gly, Ambion, Austin, TX). Blots were hybridized with [α^{32}P]dCTP-labeled DESC1 cDNA probe spanning 581 nucleotides of the DESC1 coding sequence (nucleotides 165-746), or control β-actin cDNA (CLONTECH Laboratories Inc., Palo Alto, CA), according to manufacturer's protocol. Blots were then washed in 0.5 x SSC, 0.1% SDS for 30 min. at room temperature, followed by 0.1 x SSC, 0.1% SDS for 1 hr. at 50°C with two changes of solution. The blots were then exposed to BioMax film.

As shown in Fig. 3B, expression of DESC1 was lower in squamous cell carcinoma specimens relative to matched normal specimens. Additionally, DESC1 expression was not detected in any metastatic nodal tissue samples, with the exception of minimal expression in a pharyngeal carcinoma metastatic to regional neck nodes.

Example 3

Specimens for analysis comprising primary carcinoma, metastatic node and matched normal tissue were obtained from an individual who presented with a primary squamous cell carcinoma of the tongue which was metastatic to regional neck nodes. The mRNA extraction, amplification and analysis for expression of DESC1 was conducted as in Example 1. As shown in Figure 2, DESC1 was expressed at a high level in normal tissue sample but, in contrast, was expressed at a very low level in the primary tongue carcinoma and absent from the metastatic nodal tissue derived from the same individual.

Notably, little to no DESC1 PCR transcripts were detected in the tumor samples of Examples 1-3. These results show that the present method is useful for determining the levels of DESC1 mRNA in tumor tissue from individuals with squamous cell carcinoma and for distinguishing squamous cell carcinoma tissue from healthy tissue. Such method is also useful to determine whether expression is absent, present, or altered. Thus, the present

method is also useful for research purposes to determine whether therapeutic agents modulate expression of the DESC- 1 gene.

Example 4

The DESC1 gene has been cloned into mammalian expression vector pcDNA3.1/V5/His-TOPO, in both the sense and anti-sense direction. These constructs and the empty vector alone were transfected into NIH3T3 cells and stable colonies were obtained via use of the neomycin resistance gene present on the sample plasmid. Colonies obtained were counted. Total colony count for two flasks transfected with DESC1 sense construct is 128. Total colony count for two flasks transfected with anti-sense DESC1 construct is 301. Total colony count for two flasks transfected with empty vector alone is 245. Additionally, colonies from DESC1 sense construct were smaller, suggesting that the growth rate of the colonies was slower. Thus, the present invention also relates to a method altering replication or growth of host cells by introducing a polynucleotide which encodes the DESC 1 into such cells and then expressing the polynucleotide.

Example 5

Expression of DESC1 in prostate cancer specimens and cell lines.

Expression of DESC1 was assayed in specimens of human prostate cancer and prostate cancer cell lines. RT-PCR analysis of DESC1 expression was performed using PCR primers D10 and D11 previously used to assay for epxression of DESC1 in squamous cell carcinomas of the head and neck. The results show lack of DESC1 expression in 2/3 human prostate cancer xenografts grown in mice. Expression of DESC1 is also undetectable in human prostate cancer cell lines DU145 and PC3. However, expression of DESC1 can be detected in normal human prostate tissue, normal human prostate epithelial cells (PrEC cells, Clonetics) and in normal prostate cells immortalized with HPV (PZ HPV-7).

Example 6

Western Analysis of DESC1 Expression in Transfected Human Embryonal Kidney Epithelial Cells

The DESC1 cDNA was cloned into ecdysone-inducible mammalian expression vector pIND Topo TA to produce recombinant clone pDESC1/IND C4. In this clone, the DESC1 polypeptide is expressed as a fusion protein with a carboxy-terminal tag containing the V5 epitope from the paramyxovirus SV5. When expressed in mammalian cells, DESC1 can then be detected by Western analysis utilizing an antibody to the V5 epitope. PDESC1/InD C4 was transfected into human embroyonal kidney epithelial calls (293 cells) previously

engineered to express the insect ecdysone receptor. Transfection of pDESC1/InD C4 was performed using Effectene (Qiagen) transfection reagent. Expression of DESC1 from this recombinant clone was accomplished by addition of Ponasterone (a synethetic analog of ecdysone) which allows binding of the ecdysone receptor to its response element in the promoter controlling DESC1 expression according to the manufacturer's instructions (Invitrogen). 72 hours post transfection, cell lysates were made from 293 cells both treated and untreated with Ponasterone. Lysates were then run on NuPAGE acrylamide gels (Novex) and Western analysis performed utilizing a chemiluminescent Western blotting immunodetection system (Novex). The results are shown below and demonstrate presence of a polypeptide approximately 52kd in lanes 1 and 3 (ponasterone induced) but not in lanes 2 and 4 (ponasterone negative). The molecular weight of 52kd is consistent with the predicted size of the fusion polypeptide produced by DESC1 (47kd) and the fused V4/HIS Tag epitopes (5kd).

Example 7

Expression of DESC1 in COS cells, Purification of Recombinant Protein and Assay for Protease Activity

DESC1 full length cDNa was cloned into mammalian expression vector pcDNA4/HisMax (Invitrogen). In this recombinant construct, the DESC1 polypeptide is expressed as a fusion protein with an amino-terminal His tag. DNA was transfected into COS cells utilizing Superfect transfection reagent. 72 hours after transfection, cells were lysed and recombinant DESC1 polypeptide purified utilizing the Xpress protein purification system (Invitrogen) according to the manufacturer's instructions. Purification was accomplished via binding of the His tag to ProBond resin and subsequent elution of the recombinant polypeptide. Protease activity was then tested by incubation of DESC1 recombinant polypeptide in a PepTag Protease Assay. In this assay, presence of protease activity is demonstrated by proteolysis of small dye-linked peptides. Digestion of the peptides alters the size and charge, and these changes can be detected by agarose gel electrophoresis. DESC1 polypeptide was incubated with PepTag peptide C1, with sequence: Dye-Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ala-Ala-Lys. Proteolytic cleavage between the C-terminal lysine and the internal arginine yields fragments with a neutral charge which remain in the well on electrophoresis. Intact peptide has a net positive charge and migrates towards the negative electrode. Protease activity was measured according to the manufacturer's protocol (Promega). The results are shown below and demonstrate proteolytic cleavage of C1 peptide by fractions containing

DESC1 polypeptide eluted from the ProBond resin. Results show increasing activity within fractions 1-6, while peptide incubated with elution buffer alone (lane 7) shows no protease activity (peptide intact). Positive control alkaline protease demonstrates protease activity (lane 8) similar to that of DESC1 fractions.

CLAIMS

What is claimed is:

1. A method for detecting DESC1 gene expression in a sample from a subject comprising the following steps:

providing a tissue sample from the subject;

assaying for expression of DESC1 gene in said sample.

2. An isolated nucleic acid comprising a polynucleotide encoding a protein having at least 90% identity to the DESC1 protein shown in Figure 1A or B.

3. The isolated nucleic acid of claim 2 having wherein the polynucleotide encodes a mature from or a soluble form of the DESC1 protein.

4. A recombinant vector comprising the nucleic acid of claim 2.

5. A recombinant host comprising the recombinant vecotor of claim 4.

6. An isolated DESC1 protein comprising a sequence having at least 90% identity to the amino acid sequence in Figure 1A or 1B.

7. An antibody that binds specifically to the DESC1 protein.

8. The isolated DESC 1 protein of claim 6 comprising the amino acid sequence set forth in Figure 1A or 1B.

9. The isolated nucleic acid of claim 2 wherein the polynucleotide hybridizes under stringent conditions with the nucleotide sequence shown in Figure 1A or 1B.

ABSTRACT

The present invention provides a novel method for diagnosing squamous cell carcinoma or prostate cancer in a tissue sample. The method does not involve visual examination of morphology. The method comprises providing a sample from the subject and assaying for the presence, or absence or reduced level of expression of a novel gene, hereinafter referred to as the "DESC1 gene". The method comprises isolating RNA, preferably mRNA from tissue samples, and detecting the mRNA which encodes all or part of DESC1 protein. Preferably the detection comprises amplifying the mRNA, preferably by reverse transcriptase-PCR using primers specific to a region in the DESC1 gene; and detecting the presence or absence of the amplified product to determine whether DESC1 mRNA is present or absent in the tissue sample. Alternatively the detection comprises separating the RNA which encodes all or part of DESC1 protein from the total RNA, preferably by separating on an agarose gel, and detecting the mRNA encoding DESC1, preferably by using a probe specific for such mRNA. Optionally, the DESC1 mRNA when present, is also quantified using conventional techniques. The present invention also relates to polynucleotides which encode the DESC1 protein, to the DESC 1 protein, and to antibodies to the DESC1 protein. The present invention also relates to hybridization probes, and to primers useful in the method of detecting DESC1 mRNA.

Fig. 1A

A

1 tgacttggatgtagacctcgaccttcacaggactcttcattgctggttggcaatg ATG TAT CGG CCA GAT GTG 73
1 M Y R P D V 6

74 GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATC TTC ATA 133
7 V R A R K R V C W E P W V I G L V I F I 26

134 TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA CTC ACT GTT CAT TAT GTG AGA TAT AAT CAA 193
27 S L I V L A V C I G L T V H Y V R Y N Q 46

194 AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG 253
47 K K T Y N Y Y S T L S F T T D K L Y A E 66

254 TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG 313
67 F G R E A S N N F T E M S Q R L E S M V 86

314 AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG 373
87 K N A F Y K S P L R E E F V K S Q V I K 106

374 TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT 433
107 F S Q Q K H G V L A H M L L I C R F H S 126

434 ACT GAG GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA 493
127 T E D P E T V D K I V Q L V L H E K L Q 146

494 GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG 553
147 D A V G P P K V D P H S V K I K K I N K 166

554 ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT 613
167 T E T D S Y L N H C C G T R R S K T L G 186

614 CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT 673
187 Q S L R I V G G T E V E E G E W P W Q A 206

674 AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT 733
207 S L Q W D G S H R C G A T L I N A T W L 226

734 GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT 793
227 V S A A H C F T T Y K N P A R W T A S F 246

794 GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA 853
247 G V T I K P S K M K R G L R R I I V H E 266

854 AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT 913
267 K Y K H P S H D Y D I S L A E L S S P V 286

914 CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA 973
287 P Y T N A V H R V C L P D A S Y E F Q P 306

974 GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT 1033
307 G D V M F V T G F G A L K N D G Y S Q N 326

1034 CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT 1093
327 H L R Q A Q V T L I D A T T C N E P Q A 346

1094 TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT 1153
347 Y N D A I T P R M L C A G S L E G K T D 366

1154 GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC 1213
367 A C Q G D S G G P L V S S D A R D I W Y 386

1214 CTT GCT GGA ATA GTG AGC TGG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT 1273
387 L A G I V S W G D E C A K P N K P G V Y 406

1274 ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336
407 T R V T A L R D W I T S K T G I * 423

1337 tcatggaacagataacatttttttttgtttttgggtgtggaggccattttagagatacagaattggagaagacttgca 1416

1417 aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa 1471

A

Fig. 1B

```
1    tgacttggatgtagacctcgaccttcacaggactcttcattgctggttggcaatg ATG TAT CGG CCA GAT GTG  73
1                                                             M   Y   R   P   D   V    6

74   GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATG TTC ATA  133
7    V   R   A   R   K   R   V   C   W   E   P   W   V   I   G   L   V   M   F   I    26

134  TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA GTC ACT GTT CAT TAT GTG AGA TAT AAT CAA  193
27   S   L   I   V   L   A   V   C   I   G   V   T   V   H   Y   V   R   Y   N   Q    46

194  AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG  253
47   K   K   T   Y   N   Y   Y   S   T   L   S   F   T   T   D   K   L   Y   A   E    66

254  TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG  313
67   F   G   R   E   A   S   N   N   F   T   E   M   S   Q   R   L   E   S   M   V    86

314  AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG  373
87   K   N   A   F   Y   K   S   P   L   R   E   E   F   V   K   S   Q   V   I   K    106

374  TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT  433
107  F   S   Q   Q   K   H   G   V   L   A   H   M   L   L   I   C   R   F   H   S    126

434  ACT GAG GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA  493
127  T   E   D   P   E   T   V   D   K   I   V   Q   L   V   L   H   E   K   L   Q    146

494  GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG  553
147  D   A   V   G   P   P   K   V   D   P   H   S   V   K   I   K   K   I   N   K    166

554  ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT  613
167  T   E   T   D   S   Y   L   N   H   C   C   G   T   R   R   S   K   T   L   G    186

614  CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT  673
187  Q   S   L   R   I   V   G   G   T   E   V   E   E   G   E   W   P   W   Q   A    206

674  AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT  733
207  S   L   Q   W   D   G   S   H   R   C   G   A   T   L   I   N   A   T   W   L    226

734  GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT  793
227  V   S   A   A   H   C   F   T   T   Y   K   N   P   A   R   W   T   A   S   F    246

794  GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA  853
247  G   V   T   I   K   P   S   K   M   K   R   G   L   R   R   I   I   V   H   E    266

854  AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT  913
267  K   Y   K   H   P   S   H   D   Y   D   I   S   L   A   E   L   S   S   P   V    286

914  CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA  973
287  P   Y   T   N   A   V   H   R   V   C   L   P   D   A   S   Y   E   F   Q   P    306

974  GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT  1033
307  G   D   V   M   F   V   T   G   F   G   A   L   K   N   D   G   Y   S   Q   N    326

1034 CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT  1093
327  H   L   R   Q   A   Q   V   T   L   I   D   A   T   T   C   N   E   P   Q   A    346

1094 TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT  1153
347  Y   N   D   A   I   T   P   R   M   L   C   A   G   S   L   E   G   K   T   D    366

1154 GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC  1213
367  A   C   Q   G   D   S   G   G   P   L   V   S   S   D   A   R   D   I   W   Y    386

1214 CTT GCT GGA ATA GTG AGC TCG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT  1273
387  L   A   G   I   V   S   S   G   D   E   C   A   K   P   N   K   P   G   V   Y    406

1274 ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336
407  T   R   V   T   A   L   R   D   W   I   T   S   K   T   G   I   *                423

1337 tcatggaacagataacatttttttttgtttttttgggtgtggaggccatttttagagatacagaattggagaagacttgca 1416

1417 aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa                           1471
```

Fig. 1B (con't)

A

1 tgacttggatgtagacctcgaccttcacaggactcttcattgctggttggcaatg ATG TAT CGG CCA GAT GTG 73
1 M Y R P D V 6

74 GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATG TTC ATA 133
7 V R A R K R V C W E P W V I G L V M F I 26

134 TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA GTC ACT GTT CAT TAT GTG AGA TAT AAT CAA 193
27 S L I V L A V C I G V T V H Y V R Y N Q 46

194 AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG 253
47 K K T Y N Y Y S T L S F T T D K L Y A E 66

254 TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG 313
67 F G R E A S N N F T E M S Q R L E S M V 86

314 AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG 373
87 K N A F Y K S P L R E E F V K S Q V I K 106

374 TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT 433
107 F S Q Q K H G V L A H M L L I C R F H S 126

434 ACT GAG GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA 493
127 T E D P E T V D K I V Q L V L H E K L Q 146

494 GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG 553
147 D A V G P P K V D P H S V K I K K I N K 166

554 ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT 613
167 T E T D S Y L N H C C G T R R S K T L G 186

614 CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT 673
187 Q S L R I V G G T E V E E G E W P W Q A 206

674 AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT 733
207 S L Q W D G S H R C G A T L I N A T W L 226

734 GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT 793
227 V S A A H C F T T Y K N P A R W T A S F 246

794 GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA 853
247 G V T I K P S K M K R G L R R I I V H E 266

854 AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT 913
267 K Y K H P S H D Y D I S L A E L S S P V 286

914 CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA 973
287 P Y T N A V H R V C L P D A S Y E F Q P 306

974 GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT 1033
307 G D V M F V T G F G A L K N D G Y S Q N 326

1034 CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT 1093
327 H L R Q A Q V T L I D A T T C N E P Q A 346

1094 TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT 1153
347 Y N D A I T P R M L C A G S L E G K T D 366

1154 GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC 1213
367 A C Q G D S G G P L V S S D A R D I W Y 386

1214 CTT GCT GGA ATA GTG AGC TCG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT 1273
387 L A G I V S S G D E C A K P N K P G V Y 406

1274 ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336
407 T R V T A L R D W I T S K T G I * 423

1337 tcatggaacagataacatttttttttgtttttgggtgtggaggccatttttagagatacagaattggagaagacttgca 1416

1417 aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa 1471

Fig. 2

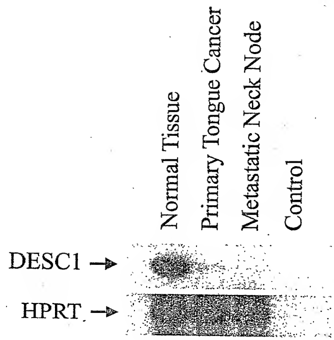

Normal Tissue
Primary Tongue Cancer
Metastatic Neck Node
Control
DESC1
HPRT

Fig. 3A

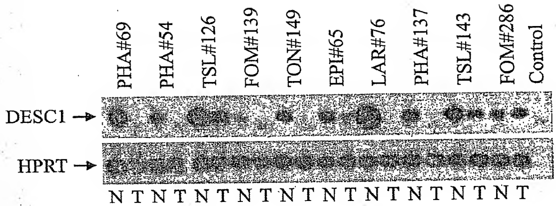

PHA#69
PHA#54
TSL#126
FOM#139
TON#149
EPI#65
LAR#76
PHA#137
TSL#143
FOM#286
Control
DESC1
HPRT
N T N T N T N T N T N T N T N T N T N T

Fig. 3B

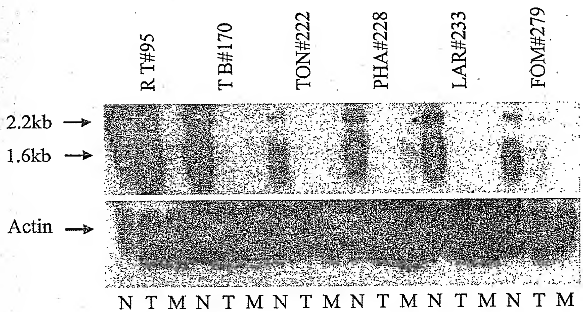

R T#95
T B#170
TON#222
PHA#228
LAR#233
FOM#279
2.2kb
1.6kb
Actin
N T M N T M N T M N T M N T M N T M

Fig. 4

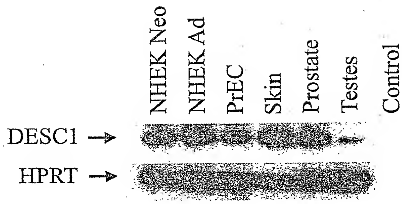

NHEK Neo
NHEK Ad
PrEC
Skin
Prostate
Testes
Control
DESC1
HPRT

Fig. 5A

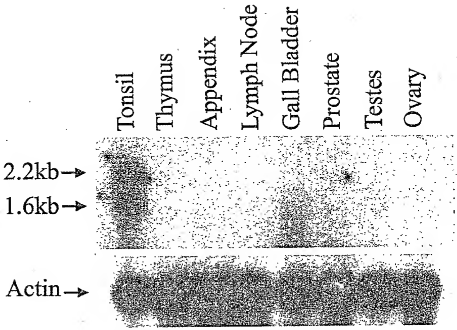
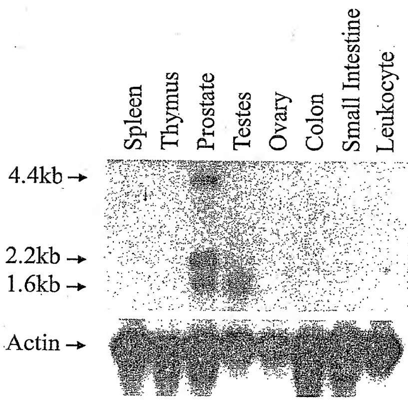

Tonsil
Thymus
Appendix
Lymph Node
Gall Bladder
Prostate
Testes
Ovary
2.2kb
1.6kb
Actin

Fig. 5B


Spleen
Thymus
Prostate
Testes
Ovary
Colon
Small Intestine
Leukocyte
4.4kb
2.2kb
1.6kb
Actin

09/674035

Fig. 6

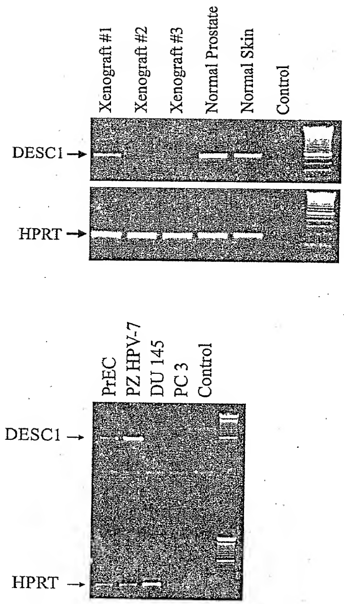
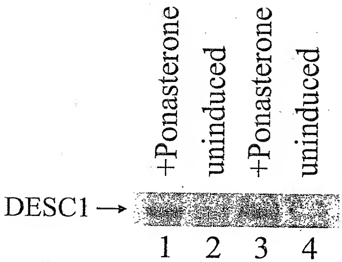
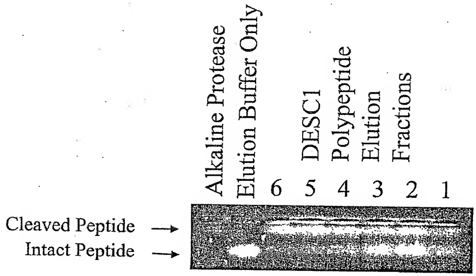


Fig. 7



09/674035

Fig. 8



09/674035
532 Rec'd PCT/PTC 25 OCT 2000

DETECTING THE EXPRESSION OF THE DESC1 GENE IN SQUAMOUS CELL CARCINOMA

This invention was made in part with government support under CA63134 and DE12704 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

Background of the Invention

Typically, morphological examination of the tissues is performed to characterize squamous cell carcinomas of the head and neck and tissues adjacent to such tumor tissue. The morphological examination usually involves the sectioning and staining of an excised tissue sample followed by microscopic examination by a cytologist or pathologist. However, visual examination does result in some errors, particularly by pathologists who do not routinely encounter such tissue samples.

Accordingly, it is desirable to have a technique which does not require visual examination of morphological characteristics to diagnose or confirm a pathologist's diagnosis of squamous cell carcinoma of the head and neck.

Summary of the Invention

The present invention provides a novel method for diagnosing squamous cell carcinoma or prostate cancer in a tissue sample. The method does not involve visual examination of morphology. The method comprises providing a sample from the subject and assaying for the presence, or absence or reduced level of expression of a novel gene, hereinafter referred to as the "DESC1 gene". The method comprises isolating RNA, preferably mRNA from tissue samples, and detecting the mRNA which encodes all or part of DESC1 protein. Preferably the detection comprises amplifying the mRNA, preferably by reverse transcriptase-PCR using primers specific to a region in the DESC1 gene; and detecting the presence or absence of the amplified product to determine whether DESC1 mRNA is present or absent in the tissue sample. Alternatively the detection comprises separating the RNA which encodes all or part of DESC1 protein from the total RNA, preferably by separating on an agarose gel, and detecting the mRNA encoding

DESC1, preferably by using a probe specific for such mRNA. Optionally, the DESC1 mRNA when present, is also quantified using conventional techniques.

The present invention also relates to polynucleotides which encode the DESC1 protein, to the DESC 1 protein, and to antibodies to the DESC1 protein. The present invention also relates to hybridization probes, and to primers useful in the method of detecting DESC1 mRNA.

Brief Description of the Figures

FIGURE 1A is the nucleotide sequence, SEQ.ID.NO. 1, of a cDNA which encodes a DESC1 protein, with the predicted amino acid sequence of the amino acid sequence, SEQ. ID. NO. 2 encoded by the nucleotide sequence. Putative initiation and termination codons are boxed, as are residues predicted to represent conserved amino acids of the catalytic triad. Suggested location of catalytic cleavage site is shown by vertical arrow. Consensus polyadenylation signals are double underlined. The predicted hydrophobic transmembrane sequence or signal peptide is located at about amino acids 18-37 and is underlined.

FIGURE 1B is an alternate nucleotide sequence, SEQ.ID.NO. 3, of a cDNA which encodes a DESC1 protein, with the predicted amino acid sequence of the amino acid sequence, SEQ. ID. NO. 4 encoded by the nucleotide sequence.

Figure 2. DESC1 expression in normal tissue and metastatic neck node used for RDA analysis and in primary carcinoma from the same patient. RT-PCR analysis. Primers utilized: D10, D11. Size of RT-PCR product, 555 bp.

Figure 3. Expression of DESC1 in squamous cell carcinoma of the head and neck (SSCHN) and matched normal tissues. A, RT-PCR analysis of DESC1 expression in SCCHN and matched normal tissue. Primers utilized: D3, D4. Size of RT-PCR product 149 bp. Low molecular weight band seen in negative control lane and some sample lanes represents unincorporated [α^{32}P]dCTP. *B*, Northern analysis of DESC1 expression in SCCHN, matched normal tissue and metastatic neck node. PHA, pharynx; TSL, tonsil; FOM, floor of mouth; TON, tongue; EPI, epiglotis; LAR, larynx; R T, retromolar trigone; T B, tongue base.

Figure 4. Expression of DESC1 in epithelial cell lines and human tissues. RT-PCR analysis using total RNA derived from epithelial cell lines and human tissues. Primers utilized, D10 and D11.

Figure 5. Expression of DESC1 in multiple human tissues. Northern analysis using *A*

,total RNA (Human Normal Tissue Blot III) and *B*, polyadenylated RNA (Human Multiple Tissue Northern Blot II).

Detailed Description of the Invention

A novel gene has been discovered which is expressed in significant levels in epithelial derived tissues, specifically epithelial tissues of the head, neck, oral mucosa, tonsils, prostate, testes, and skin in healthy individuals, i.e. individuals who do not have squamous cell carcinoma or prostate carcinoma. However, in tumor tissue samples taken from patients with squamous cell carcinoma, particularly of the head and neck, the expression of the DESC1 gene is absent or significantly reduced. The differential expression permits the identification of squamous cell carcinoma of the head and neck.

Similarly, expression of the DESC1 gene is reduced or absent in prostate carcinoma and, thus, permits identification of prostate carcinoma.

Polynucleotides

The present invention provides isolated polynucleotides which encode a DESC1 protein. One embodiment of a polynucleotide which encodes a DESC1 protein is shown in Fig. 1A, another embodiment is shown in Fig. 1B

Due to the known degeneracy of the genetic code wherein more than one codon can encode the same amino acid, a DNA sequence may vary from that shown in Fig 1A and still encode a DESC1 protein having the amino acid sequences shown in Fig. 1A. Similarly, a DNA sequence may vary from that shown in Fig. 1A and 1B and still encode the amino acid sequence shown in Fig. 1B.

The present invention also encompasses polynucleotides having sequences that are capable of hybridizing to the nucleotide sequences of Figs 1A and 1B under stringent conditions, preferably highly stringent conditions. Hybridization conditions are based on the melting temperature TM of the nucleic acid binding complex or probe, as described in Berger and Kimmel (1987) Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 152, Academic Press. The term "stringent conditions, as used herein, is the "stringency" which occurs within a range from about Tm-5 (5° below the melting temperature of the probe) to about 20° C below Tm.. "Highly Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20

μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.2x SSC at about 65 degree C. As recognized in the art, stringency conditions can be attained by varying a number of factors such as the length and nature, i.e., DNA or RNA, of the probe; the length and nature of the target sequence, the concentration of the salts and other components, such as formamide, dextran sulfate, and polyethylene glycol, of the hybridization solution. All of these factors may be varied to generate conditions of stringency which are equivalent to the conditions listed above.

Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lower stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2 M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

The present invention also encompasses alleles of the DESC1 protein encoding sequences. As used herein, the term an "allele" or "allelic sequence" is an alternative form of an DESC1 encoding sequence. The allele may result from one or more mutations in the DESC1 encoding sequence. Such mutations typically arise from natural addition, deletion of substitution of nucleotides in the open reading frame sequences. Any gene which encodes a DESC1 protein may have none, one, or several allelic forms. Such alleles are identified using conventional techniques, such as, for example, screening libraries with probes.

The present invention also encompasses altered polynucleotides which encode a DESC1 protein. Such alterations include deletions, additions, or substitutions. Such alterations may produce a silent change and result in a DESC1 protein having the same amino acid sequence as

the DESC1 protein encoded by the unaltered polynucleotide. Such alterations may produce a nucleotide sequence possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eucaryotic host may be incorporated into the nucleotide sequences shown in Figs 1A and 1B to increase the rate of expression of the polypeptides encoded by such sequences. Such alterations, conventionally, are accomplished using site-directed mutagenesis.

The polynucleotides are useful for producing DESC1 protein. For example, an RNA molecule encoding a DESC1 protein is used in a cell-free translation systems to prepare such polypeptide. Alternatively, a DNA molecule encoding a DESC1 protein is introduced into an expression vector and used to transform cells. Examples of expression vectors are chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40, bacterial plasmids, phage DNAs; yeast plasmids, vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, baculovirus, and retrovirus. The DNA sequence is introduced into the expression vector by conventional procedures.

Accordingly, the present invention also relates to recombinant constructs comprising one or more of the polynucleotide sequences. Examples of constructs are vectors, such as a plasmid, phagemid, or viral vector, into which a sequence that encodes the DESC1 protein has been inserted. In the expression vector, the DNA sequence which encodes the DESC1 protein is operatively linked to an expression control sequence, i.e., a promoter, which directs mRNA synthesis. Representative examples of such promoters, include the LTR or SV40 promoter, the *E. coli* lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or in viruses. The promoter may also be the natural promoter of the DESC1 encoding sequence. The expression vector, preferably, also contains a ribosome binding site for translation initiation and a transcription terminator. Preferably, the recombinant expression vectors also include an origin of replication and a selectable marker, such as for example, the ampicillin resistance gene of *E. coli* to permit selection of transformed cells, i.e. cells that are expressing the heterologous DNA sequences. The polynucleotide sequence encoding the DESC1 protein is incorporated into the vector in frame with translation initiation and termination sequences.

The polynucleotides encoding a DESC1 protein are used to express recombinant protein using conventional techniques. Such techniques are described in Sambrook, J. et al (1989)

Molecular Cloning A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y. and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John wile & Sons, New York, NY.

Polynucleotides encoding a DESC1 protein or fragments thereof, are also useful for designing hybridization probes for isolating and identifying cDNA clones and genomic clones encoding a DESC1 protein or allelic forms thereof. Such hybridization techniques are conventional. The sequences that encode the DESC1 proteins or fragments thereof, are also useful for designing primers for polymerase chain reaction (PCR), a technique useful for obtaining large quantities of cDNA molecules that encode the DESC1 proteins.

Also encompassed by the present invention, are single stranded polynucleotides, hereinafter referred to as antisense polynucleotides, having sequences which are complementary to the DNA and RNA sequences which encode the DESC1 proteins. The term "complementary" as used herein refers to the natural binding of the polynucleotides under permissive salt and temperature conditions by base pairing.

Isolated polynucleotides encoding a DESC1 protein are also useful as chromosome markers to map related gene positions. DESC1 polynucleotide probes are preferably labeled with radioisotopes, fluorescent labels or enzymatic labels.

Polynucleotides encoding an DESC1 protein are useful to detect DESC1 gene expression in biopsied tissue samples. DESC1 polynucleotides or fragments thereof are also useful as probes or primers to identify tissues or cells in which the corresponding DESC1 gene transcript is expressed.

Proteins

The DESC1 protein possesses four regions of conserved homology with members of the serine protease gene family. The sequence identity between DESC1 protein and the serine protease, Human Airway Trypsin-like Protease (HAT)(Fig. 1B) at the amino acid level is 38% overall and 51% when the serine protease catalytic domain only is compared (DESC1 residues 191-422). This information suggests that DESC1 protein is a novel member of the serine-protease gene family.

DESC 1 protein has the following conserved domains: (a) a predicted hydrophobic transmembrane domain located at about amino acids 18-37; (b) a predicted catalytic cleavage site located at about amino acids 190-191; and (c) a predicted catalytic domain located at about

amino acids 191-422, containing conserved residues comprising the serine protease catalytic triad at about amino acids 231 (histidine), 276 (aspartic acid) and 372 (serine). As shown in Figure 1, the predicted mature protein encompasses about 422 amino acids, while the predicted secreted and cleaved form of DESC1, which may be membrane-bound or soluble, encompasses about 232 amino acids (residues 191-422).

DESC1 may, similar to HAT, be a transmembrane serine protease possessing an extracellular COOH-terminal catalytic region. Accordingly, DESC1 protein may be used to cleave naturally occurring substrate proteins and by amino acid substitutions, to cleave proteins which are substrates of other serine proteases. Thus DESC1 polypeptides can be used to cleave a peptide for usage in microsequencing or for peptide mapping of proteins. DESC1 serine protease activity may be assayed utilizing standard methodologies used to demonstrate the activity of other serine proteases, as described for example by Smyth et al., J. Biol. Chem., 267: 24418-24425 and utilizing commercially available serine protease substrates including, but not limited to, Benzoyl-prolyl-phenyl-alanyl-arginine-4-nitril-anilide acetate; Tosyl-glycyl-prolyl-lysine-4-ntranilide acetate; Carbobenzoxy-valyl-glycyl-arginine-4-nitril-analide acetate and N-Methoxycarobonyl-D-norleucyl-glycl-L-arginine-4-nitranilide acetate (Boehringer Mannheim). Additionally, assay of serine protease activity of DESC1 can be utilized to identify inhibitors of DESC1 activity, by addition of known protease inhibitors to the assay, such as alpha2-macroglobulin, 2-(2-Aminoethyl)-benzyensulfonyl fluoride hydrochloride and Leupeptin (Boehringer Mannheim).

The term DESC1 protein in addition to encompassing the amino acid sequences of the reference amino acid sequences shown in Figures 1A and 1B, also encompasses variant DESC1 proteins whose amino acid sequence is similar to one of the reference amino acid sequences, but does not have 100% identity with the reference amino acid sequences. Such variant DESC1 protein has an altered sequence in which one or more of the amino acids is deleted or substituted, or one or more amino acids are inserted, as compared to the reference amino acid sequence. Such variant DESC1 proteins have an amino acid sequence which is at least 90% identical, preferably, at least 95% identical, more preferably at least 98% identical, most preferably at least 99% identical to the reference amino acid sequence. Sequences which are at least 90% identical have no more than 5 alterations, i.e. any combination of deletions, insertions or substitutions, per 100 amino acids of the reference amino acid sequence. Percent identity may be determined by

comparing the amino acid sequence of the variant DESC1 protein with the reference sequence using MEGALIGN project in the DNA STAR program. The variant sequences and reference sequences are aligned for identity calculations using the method of the software basic local alignment search tool in the BLAST network service (the National Center for Biotechnology Information, Bethesda, MD) which employs the method of Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403-410. Identities are calculated, for example, by the Align program (DNAstar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are not ignored when making the identity calculation.

While variant DESC1 proteins have non-conservative amino acid substitutions, it is preferred that variant DESC1 proteins have the conservative amino acid substitutions. In conservative amino acid substitutions, the substituted amino acid has similar structural or chemical properties with the corresponding amino acid in the reference sequence. By way of example, conservative amino acid substitutions involve substitution of one aliphatic or hydrophobic amino acids, e.g. alanine, valine, leucine and isoleucine, with another; substitution of one hydroxyl-containing amino acid, e.g. serine and threonine, with another; substitution of one acidic residue, e.g. glutamic acid or aspartic acid, with another; replacement of one amide-containing residue, e.g. asparagine and glutamine, with another; replacement of one aromatic residue, e.g. phenylalanine and tyrosine, with another; replacement of one basic residue, e.g. lysine, arginine and histidine, with another; and replacement of one small amino acid, e.g., alanine, serine, threonine, methionine, and glycine, with another.

Preferably, the variant DESC1 protein is immunoreactive with antibodies that bind to the reference DESC1 protein. Guidance in determining which amino acid residues may be substituted, inserted or deleted without abolishing immunoreactivity of the variant protein with an antibody specific for the respective reference protein are found using computer programs well known in the art, for example, DNASTAR software.

The present invention also relates to fusion proteins comprising a DESC1 protein and a tag, i.e., a second protein or one or more amino acids, preferably from about 2 to 65 amino acids, more preferably from about 34 to about 62 amino acids, which are added to the amino or carboxy terminus of the DESC1 PROTEIN. Typically, such additions are made to stabilize the fusion protein or to simplify purification of an expressed recombinant form of the corresponding

DESC1 protein Such tags are known in the art. Representative examples of such tags include sequences which encode a series of histidine residues, the epitope tag FLAG, the Herpes simplex glycoprotein D, beta-galactosidase, maltose binding protein, or glutathione S-transferase.

The present invention also encompasses DESC1 proteins in which one or more amino acids, preferably no more than 10 amino acids, are altered by post-translation processes or synthetic methods. Examples of such modifications include, but are not limited to, acetylation, amidation, ADP-ribosylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or a lipid, cross-linking gamma-carboxylation, glycosylation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, sulfation, and transfer-RNA mediated additions of amino acids to proteins such as arginylation and ubiquitination.

The DESC1 protein and fragments thereof, particularly extracellular fragments thereof, are useful as immunogens to produce antibodies immunospecific for the DESC1 protein. The term "immunospecific" means the antibodies have substantially greater affinity for the DESC1 protein than for other proteins. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, and Fab fragments. Polyclonal antibodies are generated using conventional techniques, such as by administering the DESC1 protein or fragment thereof to a host animal. Depending on the host species, various adjuvants are preferably used to increase immunological response. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin, and Corynebacterium parvum are especially preferable). Conventional techniques are also used to collect blood from the immunized animals and to isolate the serum and/or the IgG fraction from the blood.

For preparation of monoclonal antibodies, conventional hybridoma techniques are used. Such antibodies are produced by continuous cell lines in culture. Suitable techniques for preparing monoclonal antibodies include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV hybridoma technique.

Various immunoassays may be used for screening to identify antibodies having the desired specificity. These include conventional techniques which involve competitive binding or immunoradiometric assays and typically involve the measurement of complex formation between the DESC1 protein and the antibody.

Antibodies to the DESC1 protein

Antibodies which are specific for and bind to the DESC1 protein or the extracellular domain of the DESC1 protein, are useful research tools for identifying tissues that contain reduced levels of the DESC1 protein and also for purifying the DESC1 protein, from cell or tissue extracts, or medium of cultured cells, or partially purified preparations of intracellular and extracellular protein. Such purification is accomplished by conventional techniques such as by affinity chromatography.

Method of Assaying for DESC1 mRNA in Tissue Samples

Expression of the DESC1 transcript in a tissue sample or cell sample is determined using conventional procedures including, but not limited to DNA-RNA hybridization or PCR amplification.

A sample of the subject's tissue is obtained from a site which is suspected as being a tumor site. Preferably, one or more tissue samples from the area adjoining or preferably distal to the putative tumor site are also obtained from the subject. More preferably, samples are also obtained from matched normal, unaffected epithelial tissue of the subject. Preferably, all tissue samples are snap-frozen in liquid nitrogen immediately following resection.

RNA is isolated from the tissue samples by conventional techniques such as a TRIzol extraction procedure see for example, Gramza et. al. "Efficient Method for Preparing Normal and Tumor Tissue for RNA Extraction" BioTechniques, volume 18, page 218 (1995) which is fully incorporated herein by reference.

The presence of DESC1 mRNA is detected by RT-PCR technology using a forward primer that anneals to a region on the antisense strand of the DESC1 gene and reverse primer which anneal to a region on the sense strand of the DESC1 gene. Preferably, the forward and reverse primers anneal to regions of the DESC1 gene which are separated by between 149 to 1471 base pairs, more preferably 300 to 400 base pairs. Preferably, the primers comprise 18-36 nucleotides, more preferably 22-31 nucleotides. Preferably, the primers have a G+C content of 40% or greater. More preferably the forward and reverse primers comprise the following sequences, respectively:

DESC1 antisense , D11, 5'-TGCATCAAGCAAACAGTTTATTGAGATC-3';

DESC1 sense, D10, 5'CCTGTTCCCTACACAAATHCAGTAC-3'.

The size of the PCR product produced using such primers is 555 bp.

Optionally, a normalization standard, such as a housekeeping gene, is included in the PCR reaction. The expression of the preferred housekeeping gene is the same level in normal squamous cells and in cells derived from squamous cell carcinoma tumor tissue. For example, suitable housekeeping genes are HPRT or genes encoding actin or tubulin. Primers to the normalization standard are selected such that the length of the PCR amplification product of the normalization standard will vary from the length of the PCR amplification product of DESC1 mRNA to allow separation of the two PCR amplification products on an agarose gel.

Preferably, an internal standard comprising a double-stranded nucleic acid fragment which competes with the DESC1 gene for the primers is added to the PCR reaction mixture. Preferably, the PCR product that results from amplification of the internal standard is of a different size from that of the PCR product that results from amplification of the DESC1 mRNA to enable separation of the two products on an agarose gel.

Optimum cycle number for PCR amplification is preferably pre-determined for each primer set using a mixture of RT reactions from ten random samples selected from specimens of tumor RNA and matched normal RNA. This step is necessary to ensure that PCR amplification remains in the linear range and that production of PCR product does not plateau. Under the reaction conditions used, the quantity of PCR product is directly proportional to the amount of radioactivity incorporated into the DNA. This method allows a comparative analysis of gene expression between samples through direct comparison of radioactivity incorporated into each PCR product.

Preferably, the presence of the primer-specific PCR product is detected by separating the PCR products on an agarose gel. The presence of the primer-specific product is detected by ethidium bromide staining of the agarose gel. More preferably, the primer-specific product contains alpha-^{32}P-deoxynucleotide as a result of incorporating alpha-^{32}-p-deoxynucleotide into the PCR reaction mixture. Detection of such PCR product is accomplished by measuring the amount of radiolabeled deoxynucleotide incorporated into the PCR product by gel scanning using autoradiograms, or by liquid scintillation counting of excised portions of the gel. Band intensity of DESC1 RT-PCR product is then compared and intensity is directly proportional to the level of expression of DESC1 RNA in the original tissue sample.

Alternatively the mRNA is extracted, separated, preferably on an agarose gel, and the mRNA encoding DESC1 is detected preferably using a probe specific for such mRNA.

DESC1 RNA is expressed in normal human epithelial tissue. DESC1 RNA is also expressed in normal human epidermal keratinocytes undergoing exponential growth in tissue culture (NEHK cells; Clonetics, San Diego, CA). DESC1 RNA expression is reduced or absent in squamous cell carcinoma (SCC). Thus, in one embodiment, reduced DESC1 expression in neoplastic tissue of epithelial origin may be considered to be a diagnostic indicator of SCC.

Isolation and Sequence Analysis of DESC1 cDNA

Representational Difference Analysis (RDA) was performed on mRNA isolated from the normal oral buccal mucosa and from an squamous cell carcinoma -positive metastatic neck node from an individual who presented with a primary squamous cell carcinoma of the tongue which was metastatic to regional neck nodes. Representational Difference Analysis was performed with carcinoma RNA as driver in the reaction, allowing selection of genes expressed in normal tissue but not in tumor tissue. Representational Difference Analysis (RDA) was performed by utilizing PCR-Select cDNA subtraction methodologies (CLONTECH, Palo Alto, CA) as described in the manufacturer's protocol. 0.5% of the Representational Difference Analysis final reaction was subject to PCR amplification. The PCR amplification products thus obtained were cloned directly into mammalian expression vector pCMV-Script (Stratagene, La Jolla, CA).

All recombinant clones were screened for inserts by PCR analysis and positive clones subjected to sequence analysis using vector-specific T3 primers. BLASTN sequence analysis was then performed, using the GenBank sequence database. One recombinant clone designated "C35", carrying a 581 bp insert, possessed an open reading frame spanning the full length of the clone. The putative gene represented by this clone was designated Differential Expressed in Squamous Cell Carcinoma Gene 1 (DESC1). The C35 clone was devoid of consensus polyadenylation signals. In order to obtain the 3' end of the gene, 3'RACE analysis was performed on the remaining normal tissue mRNA used previously for RDA analysis. Sequence analysis of 3'RACE products allowed identification of two consensus polyadenylation signal sequences separated by 633 bp (Fig. 1A). In order to obtain additional 5' sequence, 5'RACE was also performed.

A full-length DESC1 cDNA clone (pDESC1) was constructed following analysis of DESC1 sequence information obtained from overlapping pCMV-Script, 5' and 3' RACE clones. pDESC1 was generated by RT-PCR amplification of normal skin RNA (Invitrogen, Carlsbad,

CA) utilizing DESC1 primers D11 and D12. primers D11 and D12 have the following sequence:
D11, 5'-TGCATCAAGCAAACAGTTTATTGAGATC-3';
D12, 5'TGACTTGGATGTAGACCTCGACCTTCAC-3'.

The PCR product was then cloned into TOPO TA cloning vector pCDNA3.1/V5/His-Topo (Invitrogen, Carlsbad, CA).The sequence of pDESC1 was determined by cycle sequencing using a Thermo Sequenase system (Amersham, Cleveland, OH), followed by electrophoresis using the CastAway sequencing system (Stratagene, La Jolla, CA) according to the manufacturer's instructions.

The full-length nucleotide sequence(SEQ ID NO:1) of one cDNA encoding DESC 1 protein is shown in Fig. 1A. An alternate full-length nucleotide sequence, SEQ.ID.NO. 3 is shown in Figure 1B. The cDNA comprises a contiguous sequence of 1461 nucleotides which encodes a predicted open reading frame of 422 or 423 amino acid residues (SEQ ID NO:2). The open reading frame begins at an N-terminal methionine located at nucleotide position 53 or 56, and ends at a stop codon at nucleotide position 1322. The predicted molecular weight of the DESC1 protein is about 44 kDa.

Tissue Expression of DESC1

In order to characterize the tissue-specific expression pattern of DESC1, Northern analysis was performed on total RNA from multiple human tissue samples and cell lines. The results are shown in Figure 5 and demonstrate a high degree of tissue specificity of DESC1 expression. Under these conditions two predominant transcripts of 2.2 kb and 1.6 kb in size were detected in tonsil tissue. Under these conditions no significant level of DESC1 expression was detected in any other tissue examined, that is thymus, appendix, lymph node, gall bladder, ovary, spleen, colon small intestine, leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney prostate and testes. However, when Northern analysis was performed with a higher degree of sensitivity, utilizing polyadenylated RNA rather than total RNA, DESC1 expression was detected in prostate and testes. A single 1.6 kb transcript is seen in testes. The three transcripts detected in prostate include a 1.6 kb transcript a 2.2 kb transcript and an additional transcript of approximately 4.4kb, which is observed exclusively in prostate tissue. In addition, expression of DESC1 was detected at a minimal level in pancreas. No expression of DESC1 was detected in thymus, appendix, lymph node, gall bladder, ovary, spleen, colon small intestine,

leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, and kidney. Thus, the DESC1 gene is strongly tissue-specific in its pattern of expression.

To determine whether DESC1 expression was epithelial-specific, the levels of DESC1 transcripts in epithelial cell lines and tissue were determined using RT-PCR analysis. The results, shown in Fig 4 established that DESC1 is expressed in neonatal (NHEKNeo) adult (NHEKAd) and prostate (PrEC) epithelial cells, confirming that the expression of the DESC1 gene is epithelial specific. Figure 4 also shows DESC1 expression in human skin and confirms expression in prostate and testes.

The RT-PCR analysis utilized primers D10 and D11, specific to the 3' end of DESC1, and produced a product that does not encompass the internal polyadenylation consensus site. This analysis allows detection of DESC1 expression in testes. Since testes exhibits only the smaller 1.6 kb transcript, the data is consistent with identification of the 1.6 kb RNA as the transcript which encodes the DESC1 polypeptide. A second 3' RACE product isolated demonstrates processing of a DESC1 RNA at the internal polyadenylation site (nucleotides 802-807). This transcript terminates at nucleotide 823 and demonstrates that the internal site is functional.

Chromosomal Mapping Using a DESC 1 probe.

Chromosomal mapping of DESC1 gene was performed using a Human/Rodent Somatic Cell Hybrid Panel from Oncor, Gaithersburg, MD, with hybridization conditions for DESC1 probe as described above. The DESC1 probe hybridized to only the lane containing human chromosome 4. Chromosomal mapping of DESC1 was additionally performed using the Genebridge 4 Radiation Hybrid Panel (Research Genetics Inc., Huntsville, AL) according to the manufacturer's instructions by PCR amplification using primers D11 and D18, with normal human placental DdnA (Sigma, St. Louis, MO) as template. D18 primer has the following sequence, 5'-GGAATAGTGAGCTCGGGAGATG-3'.

The chromosomal location of DESC1 was then determined by accessing Whitehead Institute/MIT Center for Genome Research radiation hybrid map of the human genome. DESC1 is located on the long arm of chromosome 4, positioned 20.21cR from marker WI-5548, and between markers D4S1619 and WI-7844. D4S1619 and WI-7844 have been mapped by FISH analysis to 4q12 and 4q13. Thus DESC1 is at 4q12 - 4q13 within a region about 10Mb in size

The following examples, which describe in greater detail the procedures for determining DESC1 gene expression levels in tissue samples, are intended to illustrate but not limit the procedures.

Example 1

Ten squamous cell carcinoma specimens, and matched normal tissue, selected from diverse sites in the head and neck region were obtained. All normal tissue specimens were harvested from clinically appearing normal tissue located at least 3 cm. from the tumor margin. RNA was extracted from the samples and subjected to RT-PCR analysis. of expression of DESC1 was conducted in the samples.

1.0 μg of total RNA was used for first strand cDNA synthesis in a total volume of 25 μl and reactions otherwise performed according to manufacturer's instructions (ProSTAR, Stratagene, La Jolla, CA). PCR amplification was performed in the presence of 2 units of Taq 2000 DNA polymerase (Stratagene, La Jolla, CA), with reaction conditions: 10mM Tris-HCI (pH 8.8), 50 mM KCI, 1.5 mM $MgCl_2$, 0.01% gelatin, 400 nM each primer, 200μM dNTPs, and where appropriate, 0.25μl [α^{32}P]dCTP (3000Ci/mmol) in a final volume of 25μl. Separate reactions were performed for each primer pair with reaction conditions; 96°C 3min followed by 94°C 30s, 55°C 30's, 72°C 1 min for 31 cycles (HPRT) or 33 cycles (DESC1) and a final 5 min extension at 72°C. PCR amplification of full-length DESC1 was performed utilizing the above cycling conditions, with an additional 1 min extension time for each cycle at 72°C, and using the Advantage HF PCR kit (CLONTECH Laboratories Ind, Palo Alto, CA). PCR samples were then run through 22% agarose gels and presence of amplified product and correct product size verified by ethidium bromide fluorescence in the presence of 100 bp size markers (Gibco BRL, Gaithersburg, MD). PCR products generated were then electroblotted using a Bio-Rad Semi-Dri Electroblotter SD and transferred at 12V/110 mA for 10 min. The membrane was removed and exposed to BioMax film (Eastman Kodak, Rochester, NY). Primers utilized in PCR reactions comprise: hypoxanthine phosphoribosyl transferase (HPRT) primers HPt1, 5'-GTAATGACCAGTCAACA-3' and HPRT2, 5'-CCAGCAAGCTTGCGACCTTGACCA-3' and DESC1 primers

D3, 5'-TCACTGTTCATTATGTGAGATATAATCA-3';

D4, 5'-CACCATTGATTCAAGTCTCTGGCTCAT-3';

D10, 5'-CCTGTTCCCTACACAAATGCAGTAC-3';

D11, 5'-TGCATCAAGCAAACAGTTTATTGAGATC-3';

D12, 5'-TGACTTGGATGTAGACCTCGACCTTCAC-3' and

D18, 5'-GGAATAGTGAGCTCGGGAGATG-3'.

PCR amplification of the HPRT gene was performed as a control to demonstrate equal loading and to determine integrity of RNA. Primers sets were designed by computer analysis (Oligo 4.0; NBI, Hamel, MN) of available DNA sequences for each gene and, with the exception of set D11, D18, are intron-spanning precluding PCR amplification of any residual DNA present in RNA samples. Optimum cycle number for PCR amplification was pre-determined for each primer set using a mixture of RT reactions from ten random tumor samples. This step is necessary to ensure that PCR amplification remains in the linear range and that production of PCR product does not plateau. Under reaction conditions used, quantity of PCR product is therefore directly proportional to the amount of radioactivity incorporated into the DNA.

The results, shown in Figure 3A, showed lower levels of expression of DESC1 in 9 of the 10 primary carcinomas relative to the high level of expression in matched normal tissue. It has been discovered that in non cancerous epithelial tissue, DESC1 is expressed at a high level, yet in squamous cell carcinoma, DESC1 is not expressed or only expressed at a very low level, i.e., at levels less than 10% of the levels found in matched normal tissue

Example 2

Six specimens of squamous cell carcinoma of the head and neck, matched normal tissue and metastatic regional neck nodes were obtained. The mRNA extraction was conducted as in Example 1. A northern blot containing squamous cell carcinoma specimens was generated by electrophoresis of 10 μg total RNA on a 1% glyoxal agarose gel according to manufacturer's instructions (Northern Max-Gly, Ambion, Austin, TX). Blots were hybridized with [α^{32}P]dCTP-labeled DESC1 cDNA probe spanning 581 nucleotides of the DESC1 coding sequence (nucleotides 165-746), or control β-actin cDNA (CLONTECH Laboratories Inc., Palo Alto, CA), according to manufacturer's protocol. Blots were then washed in 0.5 x SSC, 0.1% SDS for 30 min. at room temperature, followed by 0.1 x SSC, 0.1% SDS for 1 hr. at 50°C with two changes of solution. The blots were then exposed to BioMax film.

As shown in Fig. 3B, expression of DESC1 was lower in squamous cell carcinoma specimens relative to matched normal specimens. Additionally, DESC1 expression was not

detected in any metastatic nodal tissue samples, with the exception of minimal expression in a pharyngeal carcinoma metastatic to regional neck nodes.

Example 3

Specimens for analysis comprising primary carcinoma, metastatic node and matched normal tissue were obtained from an individual who presented with a primary squamous cell carcinoma of the tongue which was metastatic to regional neck nodes. The mRNA extraction, amplification and analysis for expression of DESC1 was conducted as in Example 1. As shown in Figure 2, DESC1 was expressed at a high level in normal tissue sample but, in contrast, was expressed at a very low level in the primary tongue carcinoma and absent from the metastatic nodal tissue derived from the same individual.

Notably, little to no DESC1 PCR transcripts were detected in the tumor samples of Examples 1-3. These results show that the present method is useful for determining the levels of DESC1 mRNA in tumor tissue from individuals with squamous cell carcinoma and for distinguishing squamous cell carcinoma tissue from healthy tissue. Such method is also useful to determine whether expression is absent, present, or altered. Thus, the present method is also useful for research purposes to determine whether therapeutic agents modulate expression of the DESC- 1 gene.

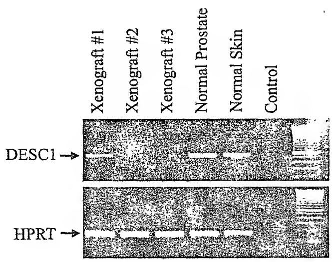
Example 4

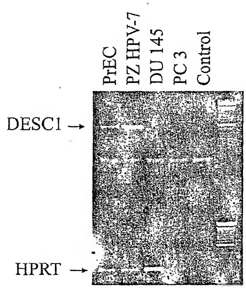
The DESC1 gene has been cloned into mammalian expression vector pcDNA3.1/V5/His-TOPO, in both the sense and anti-sense direction. These constructs and the empty vector alone were transfected into NIH3T3 cells and stable colonies were obtained via use of the neomycin resistance gene present on the sample plasmid. Colonies obtained were counted. Total colony count for two flasks transfected with DESC1 sense construct is 128. Total colony count for two flasks transfected with anti-sense DESC1 construct is 301. Total colony count for two flasks transfected with empty vector alone is 245. Additionally, colonies from DESC1 sense construct were smaller, suggesting that the growth rate of the colonies was slower. Thus, the present invention also relates to a method altering replication or growth of host cells by introducing a polynucleotide which encodes the DESC 1 into such cells and then expressing the polynucleotide.

Example 5

Expression of DESC1 in prostate cancer specimens and cell lines.

Expression of DESC1 was assayed in specimens of human prostate cancer and prostate cancer cell lines. RT-PCR analysis of DESC1 expression was performed using PCR primers D10 and D11 previously used to assay for expression of DESC1 in squamous cell carcinomas of the head and neck. The results show lack of DESC1 expression in 2/3 human prostate cancer xenografts grown in mice. Expression of DESC1 is also undetectable in human prostate cancer cell lines DU145 and PC3. However, expression of DESC1 can be detected in normal human prostate tissue, normal human prostate epithelial cells (PrEC cells, Clonetics) and in normal prostate cells immortalized with HPV (PZ HPV-7).

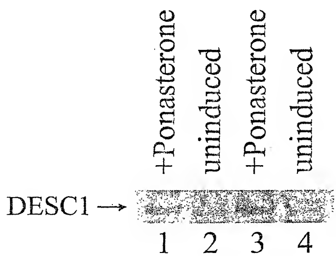




Example 6

Western Analysis of DESC1 Expression in Transfected Human Embryonal Kidney Epithelial Cells.

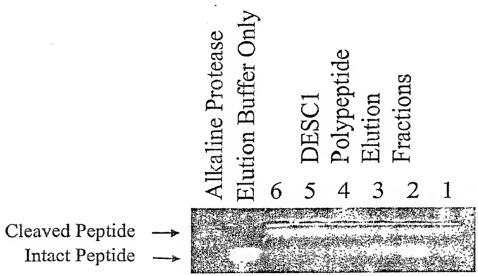
The DESC1 cDNA was cloned into ecdysone-inducible mammalian expression vector pIND Topo TA to produce recombinant clone pDESC1/IND C4. In this clone, the DESC1 polypeptide is expressed as a fusion protein with a carboxy-terminal tag containing the V5 epitope from the paramyxovirus SV5. When expressed in mammalian cells, DESC1 can then be detected by Western analysis utilizing an antibody to the V5 epitope. pDESC1/IND C4 was transfected into human embryonal kidney epitheilial cells (293 cells) previously engineered to express the insect ecdysone receptor. Transfection of pDESC1/IND C4 was performed using Effectene (Qiagen) transfection reagent. Expression of DESC1 from this recombinant clone was accomplished by addition of Ponasterone (a synthetic analog of ecdysone) which allows binding of the ecdysone receptor to its response element in the promoter controlling DESC1 expression according to the manufacturer's instructions (Invitrogen). 72 hours post transfection, cell lysates were made from 293 cells both treated and untreated with Ponasterone. Lysates were then run on NuPAGE acrylamide gels (Novex) and Western analysis performed by utilizing a chemiluminescent Western blotting immunodetection system (Novex). The results are shown below and demonstrate presence of a polypeptide of approximately 52kd in lanes 1 and 3 (ponasterone induced) but not in lanes 2 and 4 (ponasterone negative). The molecular weight of 52kd is consistent with the predicted size of the fusion polypeptide produced by DESC1 (47kd) and the fused V5/HIS Tag epitopes (5kd).



Example 7

Expression of DESC1 in COS Cells, Purification of Recombinant Protein and Assay for Protease Activity.

DESC1 full length cDNA was cloned into mammalian expression vector pcDNA4/HisMax (Invitrogen). In this recombinant construct, the DESC1 polypeptide is expressed as a fusion protein with an amino-terminal His tag. DNA was transfected into COS cells utilizing Superfect transfection reagent. 72 hours after transfection, cells were lysed and recombinant DESC1 polypeptide purified utilizing the Xpress protein purification system (Invitrogen) according to the manufacturer's instructions. Purification was accomplished via binding of the His tag to ProBond resin and subsequent elution of the recombinant polypeptide. Protease activity was then tested by incubation of DESC1 recombinant polypeptide in a PepTag Protease Assay. In this assay, presence of protease activity is demonstrated by proteolysis of small dye-linked peptides. Digestion of the peptides alters the size and charge, and these changes can be detected by agarose gel electrophoresis. DESC1 polypeptide was incubated with PepTag peptide C1, with sequence: Dye-Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ala-Ala-Lys. Proteolytic cleavage between the C-terminal lysine and the internal arginine yields fragments with a neutral charge which remain in the well on electrophoresis. Intact peptide has a net positive charge and migrates towards the negative electrode. Protease activity was measured according to the manufacturer's protocol (Promega). The results are shown below and demonstrate proteolytic cleavage of C1 peptide by fractions containing DESC1 ploypeptide eluted from the ProBond resin. Results show increasing activity within fractions 1-6, while peptide incubated with elution buffer alone (lane 7) shows no protease activity (peptide intact). Positive control alkaline protease demonstrates protease activity (lane 8) similar to that of DESC1 fractions.



CLAIMS

What is claimed is:

1. A method for detecting DESC1 gene expression in a sample from a subject comprising the following steps:

   providing a tissue sample from the subject;

   assaying for expression of DESC1 gene in said sample.

2. An isolated nucleic acid comprising a polynucleotide encoding a protein having at least 90% identity to the DESC1 protein shown in Figure 1A or B.

3. The isolated nucleic acid of claim 2 having wherein the polynucleotide encodes a mature from or a soluble form of the DESC1 protein.

4. A recombinant vector comprising the nucleic acid of claim 2

5. A recombinant host comprising the recombinant vecotor of claim 4.

6. An isolated DESC1 protein comprising a sequence having at least 90% identity to the amino acid sequence in Figure 1A or 1B.

7. An antibody that binds specifically to the DESC1 protein.

8. The isolated DESC 1 protein of claim 6 comprising the amino acid sequence set forth in Figure 1A or 1B.

9. The isolated nucleic acid of claim 2 wherein the polynucleotide hybridizes under stringent conditions with the nucleotide sequence shown in Figure 1A or 1B.

A

Fig. 1A

```
   1  tgacttggatgtagacctcgaccttcacaggactcttcattgctggttggcaatg ATG TAT CGG CCA GAT GTG   73
   1                                                           M   Y   R   P   D   V    6

  74  GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATC TTC ATA  133
   7  V   R   A   R   K   R   V   C   W   E   P   W   V   I   G   L   V   I   F   I    26

 134  TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA CTC ACT GTT CAT TAT GTG AGA TAT AAT CAA  193
  27  S   L   I   V   L   A   V   C   I   G   L   T   V   H   Y   V   R   Y   N   Q    46

 194  AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG  253
  47  K   K   T   Y   N   Y   Y   S   T   L   S   F   T   T   D   K   L   Y   A   E    66

 254  TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG  313
  67  F   G   R   E   A   S   N   N   F   T   E   M   S   Q   R   L   E   S   M   V    86

 314  AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG  373
  87  K   N   A   F   Y   K   S   P   L   R   E   E   F   V   K   S   Q   V   I   K   106

 374  TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT  433
 107  F   S   Q   Q   K   H   G   V   L   A   H   M   L   L   I   C   R   F   H   S   126

 434  ACT GAG GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA  493
 127  T   E   D   P   E   T   V   D   K   I   V   Q   L   V   L   H   E   K   L   Q   146

 494  GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG  553
 147  D   A   V   G   P   P   K   V   D   P   H   S   V   K   I   K   K   I   N   K   166

 554  ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT  613
 167  T   E   T   D   S   Y   L   N   H   C   C   G   T   R   R   S   K   T   L   G   186

 614  CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT  673
 187  Q   S   L   R   I   V   G   G   T   E   V   E   E   G   E   W   P   W   Q   A   206

 674  AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT  733
 207  S   L   Q   W   D   G   S   H   R   C   G   A   T   L   I   N   A   T   W   L   226

 734  GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT  793
 227  V   S   A   A   H   C   F   T   T   Y   K   N   P   A   R   W   T   A   S   F   246

 794  GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA  853
 247  G   V   T   I   K   P   S   K   M   K   R   G   L   R   R   I   I   V   H   E   266

 854  AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT  913
 267  K   Y   K   H   P   S   H   D   Y   D   I   S   L   A   E   L   S   S   P   V   286

 914  CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA  973
 287  P   Y   T   N   A   V   H   R   V   C   L   P   D   A   S   Y   E   F   Q   P   306

 974  GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT 1033
 307  G   D   V   M   F   V   T   G   F   G   A   L   K   N   D   G   Y   S   Q   N   326

1034  CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT 1093
 327  H   L   R   Q   A   Q   V   T   L   I   D   A   T   T   C   N   E   P   Q   A   346

1094  TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT 1153
 347  Y   N   D   A   I   T   P   R   M   L   C   A   G   S   L   E   G   K   T   D   366

1154  GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC 1213
 367  A   C   Q   G   D   S   G   G   P   L   V   S   S   D   A   R   D   I   W   Y   386

1214  CTT GCT GGA ATA GTG AGC TGG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT 1273
 387  L   A   G   I   V   S   W   G   D   E   C   A   K   P   N   K   P   G   V   Y   406

1274  ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336
 407  T   R   V   T   A   L   R   D   W   I   T   S   K   T   G   I   *                423

1337  tcatggaacagataacatttttttttgtttttgggtgtggaggccatttttagagatacagaattggagaagacttgca 1416

1417  aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa                          1471
```

09/674035

Fig. 1B

A

1 tgacttggatgtagacctcgaccttcacaggactcttcattgctggttggcaatg ATG TAT CGG CCA GAT GTG 73
1 M Y R P D V 6

74 GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATG TTC ATA 133
7 V R A R K R V C W E P W V I G L V M F I 26

134 TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA GTC ACT GTT CAT TAT GTG AGA TAT AAT CAA 193
27 S L I V L A V C I G V T V H Y V R Y N Q 46

194 AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG 253
47 K K T Y N Y Y S T L S F T T D K L Y A E 66

TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG 313
F G R E A S N N F T E M S Q R L E S M V 86

AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG 373
K N A F Y K S P L R E E F V K S Q V I K 106

TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT 433
F S Q Q K H G V L A H M L L I C R F H S 126

ACT GAG GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA 493
T E D P E T V D K I V Q L V L H E K L Q 146

GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG 553
D A V G P P K V D P H S V K I K K I N K 166

ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT 613
T E T D S Y L N H C C G T R R S K T L G 186

CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT 673
Q S L R I V G G T E V E E G E W P W Q A 206

AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT 733
S L Q W D G S H R C G A T L I N A T W L 226

734 GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT 793
227 V S A A H C F T T Y K N P A R W T A S F 246

794 GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA 853
247 G V T I K P S K M K R G L R R I I V H E 266

854 AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT 913
267 K Y K H P S H D Y D I S L A E L S S P V 286

914 CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA 973
287 P Y T N A V H R V C L P D A S Y E F Q P 306

974 GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT 1033
307 G D V M F V T G F G A L K N D G Y S Q N 326

1034 CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT 1093
327 H L R Q A Q V T L I D A T T C N E P Q A 346

1094 TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT 1153
347 Y N D A I T P R M L C A G S L E G K T D 366

1154 GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC 1213
367 A C Q G D S G G P L V S S D A R D I W Y 386

1214 CTT GCT GGA ATA GTG AGC TCG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT 1273
387 L A G I V S S G D E C A K P N K P G V Y 406

1274 ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336
407 T R V T A L R D W I T S K T G I * 423

1337 tcatggaacagataacattttttttttgttttttgggtgtggaggccatttttagagatacagaattggagaagacttgca 1416

1417 aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa 1471

A

Fig. 1B (con't)

```
1     tgacttggatgtagacctcgaccttcacaggactcttcattgctggttggcaatg ATG TAT CGG CCA GAT GTG   73
1                                                              M   Y   R   P   D   V     6

74    GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATG TTC ATA   133
7     V   R   A   R   K   R   V   C   W   E   P   W   V   I   G   L   V   M   F   I     26

134   TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA GTC ACT GTT CAT TAT GTG AGA TAT AAT CAA   193
27    S   L   I   V   L   A   V   C   I   G   V   T   V   H   Y   V   R   Y   N   Q     46

194   AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG   253
47    K   K   T   Y   N   Y   Y   S   T   L   S   F   T   T   D   K   L   Y   A   E     66

254   TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG   313
67    F   G   R   E   A   S   N   N   F   T   E   M   S   Q   R   L   E   S   M   V     86

      AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG   373
      K   N   A   F   Y   K   S   P   L   R   E   E   F   V   K   S   Q   V   I   K     106

      TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT   433
      F   S   Q   Q   K   H   G   V   L   A   H   M   L   L   I   C   R   F   H   S     126

      ACT GAG GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA   493
      T   E   D   P   E   T   V   D   K   I   V   Q   L   V   L   H   E   K   L   Q     146

      GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG   553
      D   A   V   G   P   P   K   V   D   P   H   S   V   K   I   K   K   I   N   K     166

      ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT   613
      T   E   T   D   S   Y   L   N   H   C   C   G   T   R   R   S   K   T   L   G     186

      CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT   673
      Q   S   L   R   I   V   G   G   T   E   V   E   E   G   E   W   P   W   Q   A     206

      AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT   733
      S   L   Q   W   D   G   S   H   R   C   G   A   T   L   I   N   A   T   W   L     226

7:    GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT   793
227   V   S   A   A   H   C   F   T   T   Y   K   N   P   A   R   W   T   A   S   F     246

794   GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA   853
247   G   V   T   I   K   P   S   K   M   K   R   G   L   R   R   I   I   V   H   E     266

854   AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT   913
267   K   Y   K   H   P   S   H   D   Y   D   I   S   L   A   E   L   S   S   P   V     286

914   CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA   973
287   P   Y   T   N   A   V   H   R   V   C   L   P   D   A   S   Y   E   F   Q   P     306

974   GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT   1033
307   G   D   V   M   F   V   T   G   F   G   A   L   K   N   D   G   Y   S   Q   N     326

1034  CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT   1093
327   H   L   R   Q   A   Q   V   T   L   I   D   A   T   T   C   N   E   P   Q   A     346

1094  TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT   1153
347   Y   N   D   A   I   T   P   R   M   L   C   A   G   S   L   E   G   K   T   D     366

1154  GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC   1213
367   A   C   Q   G   D   S   G   G   P   L   V   S   S   D   A   R   D   I   W   Y     386

1214  CTT GCT GGA ATA GTG AGC TCG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT   1273
387   L   A   G   I   V   S   S   G   D   E   C   A   K   P   N   K   P   G   V   Y     406

1274  ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336
407   T   R   V   T   A   L   R   D   W   I   T   S   K   T   G   I   *                423

1337  tcatggaacagataacatttttttttgtttttgggtgtggaggccatttttagagatacagaattggagaagacttgca 1416

1417  aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa                         1471
```

Fig. 2

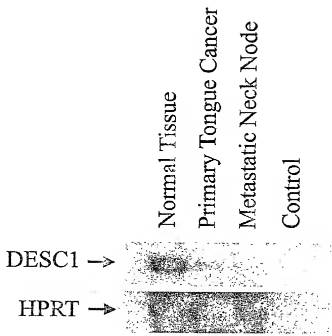
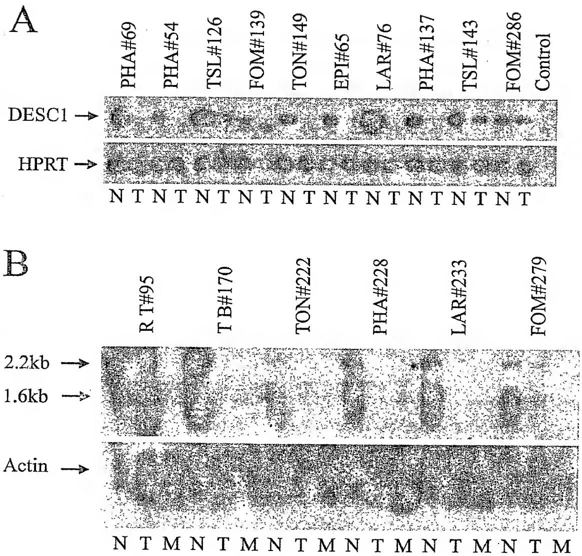

Normal Tissue
Primary Tongue Cancer
Metastatic Neck Node
Control
DESC1
HPRT

Fig. 3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N T N T N T N T N T N T N T N T N T N T
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N T M N T M N T M N T M N T M N T M

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Fig. 4

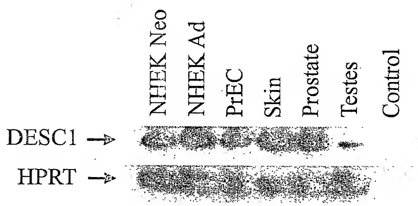

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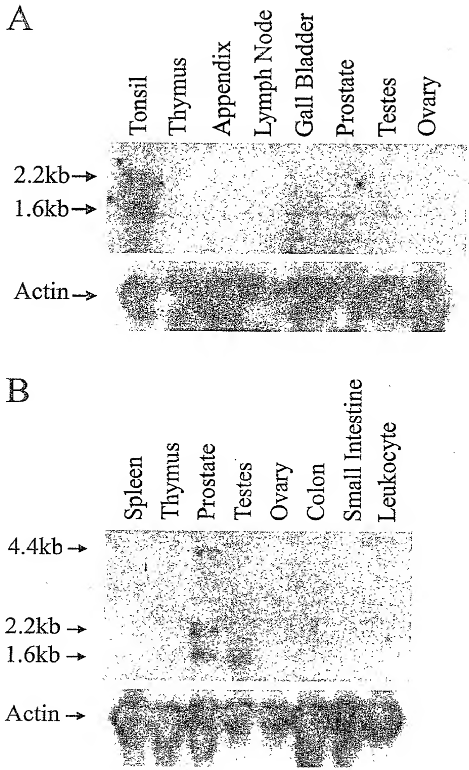
Fig. 5



CALFEE, HALTER & GRISWOLD LLP
Docket No. 22727/04078

DECLARATION
AND POWER OF ATTORNEY
ORIGINAL APPLICATION

As below named inventors, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my names.

I believe I am the original, first and sole inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DETECTING THE EXPRESSION OF THE DESC1 GENE IN SQUAMOUS CELL CARCINOMA

the specification of which

☐ is attached hereto,
☒ was filed on November 11, 1999 as Application Serial No. PCT/IB99/01818.
☐ and was amended on ______________________.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, code of Federal Regulations, §1.56(a).

I hereby appoint the following attorney(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made hereon of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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